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THE POLYNUCLEAR COUNT.

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THE POLYNUCLEAR COUNT.

THE NUCLEUS OF THE NEUTROPHIL POLYMORPHONUCLEAR LEUCOCYTE IN HEALTH AND DISEASE, WITH SOME OBSERVATIONS ON THE MACROPOLYCYTE.

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INTRODUCTION.

My personal interest in the " Arneth Count " is of many years' standing and it is with pleasure that I yield to the request of my friend, Dr. W. E. Cooke, to write a short introduction to *The Polynuclear Count*, in which he and Professor Ponder present the results of their investigations, both clinical and experimental.

I do so the more readily because I am satisfied that a critical examination of the polymorphs is of material practical value in the diagnosis and treatment of patients.

In 1904 Arneth attempted to gauge the degree of toxæmia in patients suffering from infectious diseases by noting the changes in the polymorphonuclear leucocytes. He pointed out that there were significant variations in the nuclei of the polymorph which indicated with some precision the severity of the toxæmia.

Unfortunately his method of classification was so complicated that it afforded no sure basis for other workers, with the result that the " Arneth Count " failed to find general acceptance.

Arneth's original conception was, however, sound. We have, in the structure of the nucleus of the polymorph, sure guidance as to the degree of toxæmia.

In 1914 Dr. W. E. Cooke published *The Arneth Count*, in which he gave a clear and concise description of a new method of classifying the polymorphs—a method free from ambiguity and one which can be carried out by anyone possessed of an average degree of technical skill in the preparation of blood smears.

This requires emphasis because many fail to attain confidence in their ability to make an accurate nuclear count and give up the attempt in disgust after a few abortive trials.

This failure is due, in part, to the fact that the smears are imperfectly made and stained, and also in part to ignorance of the principles on which the polymorphs are grouped.

The first is simply a question of technique, the second can be rectified in a few hours by the kindly assistance of someone familiar with this method of counting.

In his book of 1914 Dr. Cooke presented a remarkable series of records from well over three hundred patients. He demonstrated the practical value of the "Count" both in diagnosis, prognosis, and in the control of treatment. He then described clearly the necessary technique and the method of differentiating the five classes of polymorphs for the purpose of the count.

More recently, the accuracy of his findings and deductions have been amply confirmed by Professor Ponder, who approached the subject from the physiological standpoint and has thrown new and valuable light on the life-history of the polymorphonuclear leucocyte.

This method of investigation throws new light on the response to infection and is yet another index of marrow activity.

To those interested in pernicious anæmia this contribution to hæmatology gives food for reflection. It is shown, for example, that in pernicious anæmia there is a drift in the count to the right—a phenomenon unknown in any septic infection, thus opening up avenues for speculation concerning the fundamental etiology of this remarkable disease.

To hæmatologists the account of the macropolycyte

and its varieties will be of especial interest. This cell was first described by Dr. Cooke in 1925, and the recognition of its presence in any blood smear is of undoubted clinical significance.

Many practitioners of medicine find interest in hæmatology. The technique is easy, and research can be carried out independently of any laboratory. To them, an appreciation of the significance of the " Polynuclear Count " would add much to this interest.

Many fields for investigation lie open to any man prepared to devote some time to this fascinating subject.

JOHN HAY.

Liverpool,
July, 1927.

PREFACE.

ARNETH'S work on the polymorph will remain a monument to his skill and patience, but we think the time has arrived when clinical tests should be known by a descriptive title whenever possible, in preference to the author's name. For this reason only, this second edition of *The Arneth Count* is entitled *The Polynuclear Count.* We do not wish to subtract one iota from the debt we owe to Arneth.

In the 1914 edition the hope was expressed that further work on the nuclear changes in the polymorph might throw light on many hitherto inexplicable phenomena in disease.

In the following pages we attempt to summarise our experimental and clinical results from that date.

We take this opportunity to express our thanks to Mr. C. F. Hill for his invaluable help in the preparation of the photomicrographs, and to the Editors of the *British Medical Journal*, the *Journal of Experimental Physiology*, and the *Journal of the Royal Microscopical Society* for their permission to use the blocks of illustration.

W. E. C.
E. P.

Sept. 1927.

PREFACE TO *THE ARNETH COUNT.*

In the following pages an attempt has been made to place the Arneth Count on a firm basis. The interest taken in the subject is becoming greater in this country, but we are still far behind America and Germany.

Up to the present no definition of the count has been given. This, I believe, is the chief reason why the count has not been more widely used. As an adjunct to the clinical study of many diseases, the method deserves an extended trial, and by it some of the many puzzling phases of microbic infections may be elucidated.

To Drs. Lovell Gulland, John Hay, W. R. Jack, Brownlee, Claude, Ker, Pirie, Aitken, and White Robertson are due my best thanks for providing opportunities to collect material, and sending interesting specimens.

Also, I must thank Dr. Donald Fraser, Chairman, and the members of the Board of Directors of the Ochil Hills Sanatorium for the facilities provided, and the keen interest taken in this and the other researches instituted on the subject of tuberculosis.

W. E. C.

Ochil Hills Sanatorium,
Milnathort, N.B.,
1*st May*, 1914.

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THE POLYNUCLEAR COUNT

CHAPTER I.

DEFINITION.

There are several details of technique, essential in the preparation of a satisfactory blood-film, which may be mentioned.

1. **Microscope Slips.**—The ordinary 3×1 microscope slip may be used either plain or after rubbing with fine emery-paper as in opsonic index work. In either case it must be scrupulously clean and fat free. The

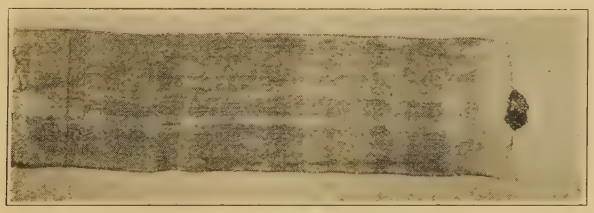

Fig. 1.

most convenient method for rendering slides fat free is by placing them in a mixture of chromic acid 1 part, strong sulphuric acid 8 parts, for 24 hours; thoroughly wash under the tap and transfer them to a mixture of alcohol and ether. They are dried with a cloth rendered fat free by boiling in sodium carbonate solution and rinsed in several changes of water, but not ironed.

2. **The Blood-film.**—The film must be thin, and it is essential that the blood should not flow to the edge of the slide. The completed film should look like fig. 1.

It has been found by experience that of all methods

the most satisfactory is the use of a piece of glass as a spreader. The spreader is $2 \times \frac{3}{4}$ inches. This is held at an angle of approximately 45 degrees to the slide on which the drop of blood has been placed. The spreader is drawn backwards until it touches the drop; it is then moved forwards and draws the blood behind it, and in a completed film leaves a flat even layer distributed about the centre of the slide. The spreader is, of course, narrower than the slide itself. The great advantage of this is that the blood does not overflow the edges of the slide. If it did, most of the leucocytes would be lost, and the film would be difficult to use for polynuclear and differential counts.[1]

3. **The Stain.**—The methods which may be used are several, each adapted to particular requirements and types of work.

For routine clinical work one of the most satisfactory stains is Giemsa, diluted 1 in 10. The film is dried in air, fixed for about ten minutes in methyl alcohol, and then stained for half an hour with the diluted stain. The film is then rinsed under the tap and air dried; after drying, it may either be examined with the oil immersion unmounted, or may be mounted in the usual way with a thin coverslip and euparal. Giemsa staining gives excellent results for the cells of man, but is not satisfactory for the cells of many animals.

If a method of universal application is required, Heidenhain's iron-alum method should be used. The dried films are fixed in saturated corrosive sublimate for about fifteen minutes, and are then transferred to the mordant, which consists of a 2·5 per cent. solution of iron alum in water. In this they may remain for any time from thirty minutes to twenty-four hours. They are then washed rapidly and placed in a 1 per cent.

[1] This spreader is made by Watson & Sons, 313 High Holborn.

hæmatoxylin in 10 per cent. alcohol; in this they remain about the same length of time as used for the mordanting. Differentiation is effected by a second immersion in the iron alum, the length of time depending on the intensity of the staining and on the degree of differentiation required. Five minutes is usually sufficient. After drying, the films may be examined either mounted or unmounted. This method gives excellent preparations, the nuclei of the polymorphs standing out almost black on an almost colourless background. The nuclear configuration can be determined with great ease. The fixation in corrosive gives less shrinkage of the cell than does methyl alcohol, with the result that the nuclear material seems more spread out in the cytoplasm ; the contrast between the nucleus and the cytoplasm gives, moreover, a very suitable condition for photomicrography. Although the method may appear rather lengthy, its use really saves much labour, as it can be applied to films in bulk. As it is applicable to the polymorphs of every type of animal, it is the ideal stain for experimental work.

Other stains which may be used for special purposes are eosin-methylene blue, carbol-pyronin green, Pappenheim's panoptic stain, and crystal violet. For notes on these methods the reader is referred to Appendix I.

We may mention here that the commonly used compound stains, such as Lieshman, Romanowsky, Jenner, etc., are not satisfactory for the purposes of the Arneth count in man, and much less satisfactory for the count in animals. With these stains it is often exceedingly difficult to determine the nuclear configuration. An extreme case of unsuitability is found in May-Grünwald, which, although quite good for general work, fails to stain the chromatin filaments between the nuclear lobes.

The film is examined with a 2-mm. objective and a

×7 or ×8 eyepiece. Care should be taken to use critical illumination, in the interests both of accuracy and of convenience. The nuclear divisions of 100 polymorphs are then counted.

The easiest and quickest way to carry out this count is to count out 100 peas, beans, or glass beads into a dish. Five little dishes are then labelled 1, 2, 3, 4, and 5, and are arranged near the microscope. When a cell of Class I. is seen one bean is put into dish 1; if a cell of Class III. is observed a bean is placed in dish 3, and so on. When the 100 beans are exhausted, 100 cells have been counted, and neither more nor less; the number of cells in each class is at once obtained by counting the beans in the respective dishes. This simple method saves a good deal of time in marking up the cells on paper. After a little practice the bean can be put in the proper dishes without the observer taking his eye from the microscope.

Observation is, of course, much facilitated by the use of a mechanical stage.

Arneth differentiates polymorphs according to their nuclear configuration and places them in five classes as follows:—

Class I.—Neutrophiles with one nucleus.

(*a*) Myelocytes (M).
(*b*) Nucleus slightly indented (W).
(*c*) Nucleus more deeply indented (T).

Class II.—Neutrophiles with two-lobed nuclei.

(*a*) Nuclei with two round pieces (2 K).
(*b*) Nuclei with two bent pieces (2 S).
(*c*) Nuclei with one round piece and one bent piece (1 K and 1 S).

Class III.—Neutrophiles with three-lobed nuclei.

(*a*) Nuclei with three round pieces (3 K).

(*b*) Nuclei with three bent pieces (3 S).
(*c*) Nuclei with two round and one bent pieces (2 K and 1 S).
(*d*) Nuclei with two bent and one round pieces (2 S and 1 K).

Class IV.—Neutrophiles with four-lobed nuclei.

(*a*) Nuclei with four round pieces (4 K).
(*b*) Nuclei with four bent pieces (4 S).
(*c*) Nuclei with three round pieces and one bent piece (3 K and 1 S).
(*d*) Nuclei with two round and two bent pieces (2 K and 2 S).
(*e*) Nuclei with one round and three bent pieces (1 K and 3 S).

Class V.—Neutrophiles with five-lobed nuclei.

(*a*) Nuclei with five round pieces (5 K).
(*b*) Nuclei with five bent pieces (5 S).
(*c*) Nuclei with four round and one bent pieces (4 K and 1 S).
(*d*) Nuclei with three round and two bent pieces (3 K and 2 S).
(*e*) Nuclei with two round and three bent pieces (2 K and 3 S).
(*f*) Nuclei with one round and four bent pieces (1 K and 4 S).

Fig. 2 is a graphic representation of Arneth's scheme taken from Gruner's *Biology of the Blood Cells*.

Looking at the classification and the diagram several objections are at once apparent. Firstly, the count is too complex to be of any service in clinical medicine. Secondly, many counts would be necessary before the observer obtained constant results. Rayevsky states that 40,000 observations were made before he became accurate, and we have no reason to doubt his statement. Thirdly, the personal factor enters into the count to

such an extent as to render the results of different

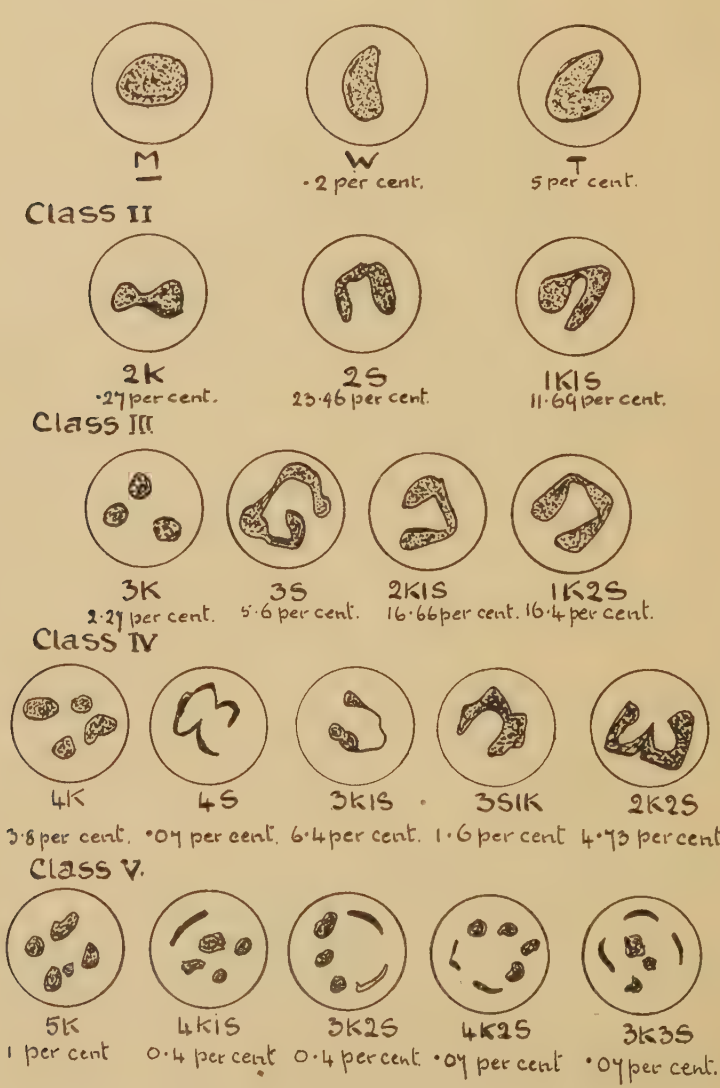

FIG. 2.

observers valueless for comparative purposes. Finally, the classification is both indefinite and unnecessarily

detailed, the distinction between nuclei with round pieces and nuclei with bent pieces being quite superfluous.

The method described by one of us in *The Arneth Count*, 1914, differed so much from Arneth's description that it has been termed the Polynuclear Count. After many thousands of observations we concluded that it was impossible to arrive at constant results by Arneth's method, and after a further experimental period the following definition was formulated :—

The polymorph with an undivided nucleus is placed in Class I. ; in Class II. is placed the cell whose nucleus has divided into two lobes ; in Class III. the cell with three lobes ; and so to Class V.

The difficulty lies in determining whether or not the nucleus has divided.

The polymorph nucleus never divides completely. There is always a fine filament of nuclear material uniting the different lobes.

> "IF THERE IS ANY BAND OF NUCLEAR MATERIAL EXCEPT THIS CHROMATIN FILAMENT CONNECTING THE DIFFERENT PARTS OF A NUCLEUS, THAT NUCLEUS CANNOT, FOR THE PURPOSES OF THE COUNT, BE SAID TO BE DIVIDED."

The following diagram explains this :—

FIG. 3.

The first figure represents a polymorph, and the five lobes its nucleus. The thick lines represent bridges of nuclear tissue connecting the lobes. Although the nucleus shows five distinct masses, the connecting threads are too thick for it to be termed anything but

a cell of Class I. The second figure represents a cell of Class II. The portions of the nucleus connected by the thick bands must be counted as one piece. The end-piece, joined only by a filament, is counted as a separate part. The third figure represents a cell of Class III., the fourth a cell of Class IV., and the fifth a cell of Class V. (Fig. 4.)

Nuclei which are free, *i.e.* without a cell body, should not be counted.

Polymorphs which have been subjected to pressure or are surrounded by red cells in rouleaux should not be counted.

In a small proportion of cells the nucleus will be found to have become folded upon itself in the preparation of the film in such a manner that it is impossible to follow the fine connections. We think it is a safe rule to place the cell in Class III. if the question is whether it is a Class III. or a Class IV. cell, into Class IV. if the doubt is about IV. and V. If there is a doubt between Class II. and III. the cell should not be counted. The reason for this rule will be seen later.

If this definition is adopted all the objections to Arneth's complex scheme are overcome, and the Polynuclear Count at once takes its place as a very valuable adjunct to clinical medicine.

The count is learned in a day or two, instead of requiring months by Arneth's method. There is a tendency to hold that much practice is necessary. With properly stained films a person of average intelligence can learn to make consistent and accurate counts in one day. We have on many occasions taught students the method by going over 100 cells with the learner; after this, one or two independent counts, checked by the teacher, constitute all the preliminary experience necessary, and hereafter it will be found

that the beginner makes no greater errors in his counts than does the expert. The making of count is, in fact, not so difficult as the making of a differential count in

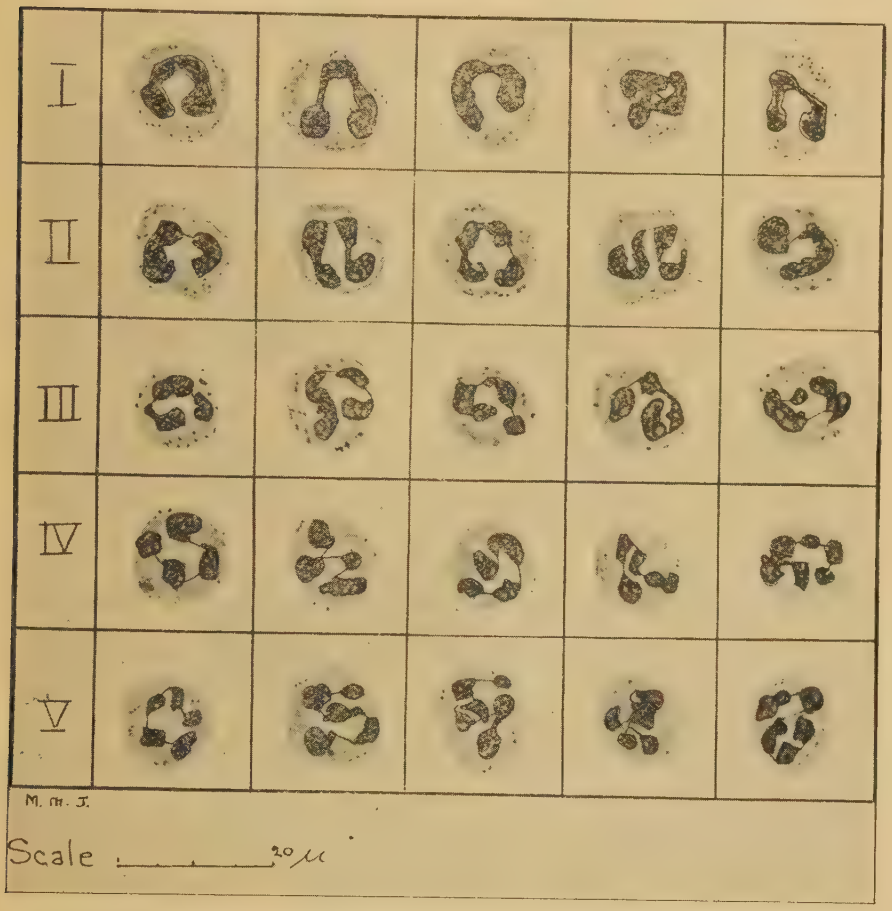

FIG. 4.—The five classes in the Polynuclear Count. If there is any band of nuclear tissue, except a chromatin filament, uniting the nuclear masses, those parts must not be considered as separate segments.

I., No. 2; II., Nos. 3 and 4; III., No. 4; and IV., No. 5, illustrate this point. In V., No. 4, the upper segment of the nucleus overlies the segment below it, but the thin chromatin filament uniting them can be seen on the left. Drawings by M. M. Johnson.

pathological blood. The only point in which the expert is at an advantage is that he can work more rapidly and is less fatigued by long series of counts.

The next question, "Is the count accurate?" is answered by the experience of those who use the method frequently. It has stood the acid test of time, the most severe test of the value of any clinical method. Further, extensive researches at the Department of Physiology in the University of Edinburgh have placed the method in an unassailable position, as the following quotation shows:—

"The sources of error are principally two—errors of sampling, and errors of judgment on the part of the observer. Plainly the errors of sampling will be smaller the greater the number of cells examined; the errors of judgment, on the other hand, will tend to increase under such circumstances, for the carrying out of an Arneth count is a fatiguing matter, and the more fatiguing the more careful the observer. The extent to which these errors influence the count is best shown by considering in detail the results on experiment.

"Ten films were made, immediately after one another, from the blood of a healthy person. These were stained, and the polymorphs examined under a 2-mm. oil-immersion. On each slide 100 cells were counted, and divided into the classes of the count, Cooke's criterion on nuclear lobulation being used. The cells were counted by 50's, so that each slide yielded two counts of 50 and one of 100. The results are shown *in extenso* in the following table, together with the calculated means [1] for the counts of 50 and for the counts of 100. All the counts were carried out by the same observer, a person with much previous experience in the method:—

[1] The weighted mean is obtained by multiplying the number of cells in Class I. by 1, those in Class II. by 2, Class III. by 3, Class IV. by 4, and Class V. by 5, the results are added and divided by the total number of cells.

Film.	I.	II.	III.	IV.	V.	Mean.	Mean for 100.
1. 1st 50	2	12	23	12	1	1·38	
2nd 50	3	13	22	11	1	1·44	2·82
2. 1st 50	3	13	20	13	1	1·46	
2nd 50	3	12	22	11	2	1·47	2·93
3. 1st 50	3	15	19	12	1	1·43	
2nd 50	3	16	21	8	2	1·40	2·83
4. 1st 50	3	16	21	8	2	1·40	
2nd 50	3	6	22	17	2	1·59	2·99
5. 1st 50	3	14	21	10	2	1·44	
2nd 50	4	10	22	13	1	1·47	2·91
6. 1st 50	3	12	20	12	3	1·50	
2nd 50	4	5	24	12	5	1·39	2·89
7. 1st 50	4	21	14	10	1	1·33	
2nd 50	1	17	22	10	0	1·41	2·74
8. 1st 50	2	14	16	15	3	1·53	
2nd 50	3	11	21	14	1	1·49	3·02
9. 1st 50	3	14	14	15	4	1·53	
2nd 50	3	14	16	15	2	1·49	3·02
10. 1st 50	3	15	16	15	1	1·46	
2nd 50	4	10	20	14	2	1·48	2·94

"The count of 1000 cells gives:—

I.	II.	III.	IV.	V.
60	260	396	247	37

"The mean of this is 2·941. The standard deviation works out at 0·946, and the error on the mean if 100 cells were counted as $\pm 0{\cdot}06$ approximately. There is no need to take account of more than the second place of decimals, as a difference of 1 unit in the second place indicates the smallest error which can be made—the

grouping of one cell in a group one too low, or one too high.

"If not 100 cells but 50 are counted, the error is $\pm 0{\cdot}09$, a slightly larger error, but one which is by no means great. This indicates that satisfactory counts can be obtained from even as few as 50 cells. It will be noted that exactly half the means of counts of 100 fall between the limits 2·88 and 3·00, while the other half fall outside these limits, as they should.

"This experiment shows the extent of the errors in the making of a normal count. Since, however, many counts are far from normal, it is of interest and importance to see what errors will be made in a count which shows a smaller mean than that of this last experiment. A similar series of ten counts of 100, and twenty counts of 50, were therefore carried out on films of blood from a person who showed a low Arneth count. The results are given in table on following page.

"The count of 1000 cells gives:

I.	II.	III.	IV.	V.
414	436	134	16	0

which gives a weighted mean of 1·752. The standard deviation is 0·74, and the probable error to which the mean is subject $\pm 0{\cdot}05$ very nearly. The limits 1·7 and 1·8 ought, therefore, to include about half the counts of 100, as they in fact do.

"If the number of cells counted is 50 instead of 100, the error to be expected is 1·414 times as large—that is, $\pm 0{\cdot}07$. By counting 50 cells instead of 100 we therefore attain a high degree of accuracy, quite sufficient for all ordinary purposes, for the error introduced by counting this small number is no greater than would be the error arising from the classifying of seven cells of Class II. in Class I.

" As a result of these experiments we are satisfied that it is unnecessary to count more than 100 cells in making a count.

" In all these counts we have used Cooke's criterion on

Film.	I.	II.	III.	IV.	V.	Mean.	Mean for 100.
1. 1st 50	23	20	7	0	0	0·84	
2nd 50	17	19	4	0	0	0·87	1·71
2. 1st 50	24	22	4	0	0	0·80	
2nd 50	15	27	8	0	0	0·93	1·73
3. 1st 50	20	25	4	1	0	0·86	
2nd 50	18	21	11	0	0	0·83	1·69
4. 1st 50	23	19	8	0	0	0·85	
2nd 50	23	19	7	1	0	0·86	1·84
5. 1st 50	17	25	7	1	0	0·92	
2nd 50	20	21	6	3	0	0·92	1·84
6. 1st 50	21	21	8	0	0	0·87	
2nd 50	24	19	7	0	0	0·83	1·70
7. 1st 50	22	20	7	1	0	0·87	
2nd 50	18	24	8	0	0	0·90	1·77
8. 1st 50	18	26	5	1	0	0·89	
2nd 50	20	23	6	1	0	0·88	1·77
9. 1st 50	26	19	5	0	0	0·79	
2nd 50	24	18	6	0	0	0·86	1·65
10. 1st 50	20	20	7	3	0	0·93	
2nd 50	21	18	9	2	0	0·92	1·85

nuclear lobulation. Thus ' if there is any band of nuclear tissue except a chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purpose of the count, be said to be divided.' This condition, as laid down by Cooke, is very different from

the original condition of Arneth, for Arneth divides the polymorphs into classes according as they contain 'one or more nuclei,' by which is meant one or more nuclear lobes. This classification does not define when a nucleus may be considered as divided, and is unfortunate in that it allows the personal factor to assert itself unduly.

"The difference which arises when a count is made, (*a*) by Cooke's method, and (*b*) by Arneth's method, is shown in the following two cases:—

		I.	II.	III.	IV.	V.	Mean.
Case 1.	Cooke	40	24	15	1	0	1·77
	Arneth	9	32	49	9	1	2·61
Case 2.	Cooke	31	46	20	3	0	1·95
	Arneth	16	59	14	8	3	2·23

"These results show how important it is to retain Cooke's classification, for if the cells be divided simply according to the number of nuclear masses seen, and not according to the lobes divided except for filaments, the figures for the count become much higher."

It will be plain from this passage that a very considerable degree of accuracy may be expected from the figures of the count. Such accuracy may, however, be obtained after even quite a short experience, and it is an excellent plan for the beginner to check his own results in a manner similar to that described above, in order to ensure that sufficient skill has been acquired.

The count is written as percentages of cells in the various classes:—

I.	II.	III.	IV.	V.
10	25	47	16	2

Whether the distribution of the figures in the various classes is expressed as an index, or otherwise, is a matter for personal consideration.

The simplest method is to draw a line between Classes II. and III., and speak of I. and II. as the left-handed columns and III., IV., and V. as right-handed.

For example:

I.	II.	III.	IV.	V.
10	25	47	16	2
35		65		

Bushnell and Treuholtz suggested the following index. The cells of Classes I. and II. and half those in Class III. are added together, thus making the index the percentage of the lower half of the picture to the whole.

Their index in the above example would be:

10 25 $\frac{1}{2}$ of 47 = 58·5

If we really want to express the distribution of the cells by a single figure the weighted mean is the best method.

The example above gives a mean of 2·75. A case of advanced pulmonary tuberculosis having a polynuclear count of:

I.	II.	III.	IV.	V.
52	34	14	0	0

has a mean of 1·62.

The weighted mean is a very sensitive measure of the state of the count, for unit difference in the second place of decimals means that one cell has been moved to the class either below or above it. It is, moreover, rather more sensitive to the numbers in the higher classes (III., IV., and V.) than to those in the lower classes; this is an advantage in practice, for in most abnormal states the first change that appears consists in a diminution in the numbers to which the mean is most sensitive.

The lower the weighted mean, the greater is the deviation to the left.

The index suggested on the basis of Hamilton Black's findings regarding the supposed phagocytic power of the cells of the various classes has rightly fallen into disuse, as it appears to have no foundation in fact.

CHAPTER II.

THE POLYNUCLEAR COUNT IN HEALTH.

A "NORMAL" in any particular branch of medicine is notoriously difficult to define, and the polynuclear count is not an exception. Our "Health Standard" is the average of ninety persons of both sexes between the ages of twelve and fifty-five. The greatest possible care was taken to exclude any focus of infection at the time the blood was taken, and for two months previously. The teeth, throat, nose, ears, chest, abdomen, and the urine were examined, and in many cases a radiograph was taken of the chest and teeth.

The average of these counts is:

I.	II.	III.	IV.	V.
10	25	47	16	2

The table on page 18 gives typical normal counts. For an extended list of figures, as well as for a detailed analysis of the normal state, the reader should refer to Appendix II.

Normal counts vary from about 20 to about 40 in the first two classes. An excellent criterion of normality is that the weighted mean of a count shall not be less than 2·4, although a rougher and less reliable guide of less than 40 cells in the first two classes may be substituted for most purposes (see Appendix II.).

It will immediately suggest itself to the reader that the remarkable constancy in the number of cells in the various polynuclear classes in health must have some physiological explanation, and that in the absence of

this explanation the count can be used only empirically and without any real appreciation of its meaning and significance. Until recently the mechanism by which the polynuclear count is maintained at its normal figure has been obscure; we now know, however, that the figures which appear in the various classes of the count are closely connected with the stages in the life-history of the polymorph, and that every variation in

I.	II.	III.	IV.	V.
9	21	53	16	1
7	29	47	16	1
14	24	45	16	1
8	20	51	18	3
7	29	47	14	3
9	30	38	21	2
12	27	38	20	3
8	21	47	22	2
11	25	47	16	1
18	22	44	14	2
8	30	39	21	2
16	20	45	18	1
3	31	46	18	2
7	31	40	20	2
5	18	49	25	3
10	18	55	15	2
4	23	48	22	3
7	29	50	14	0

the production or destruction of these cells is mirrored in the state of the count.

In principle the method used for the study of the problem is a very simple one. In any animal there is continual production of young polymorphs and continual removal of old ones; the states in the blood-stream at any moment represents a kind of equilibrium between this production and removal. It happens that by the injection of certain substances, of which thyroid extract

is one, we can disturb the state of equilibrium by greatly increasing the production of young cells; we can then follow from time to time the way in which the recovery from the disturbance proceeds. In this way, as will be seen from the individual examples which we shall consider below, we are able to elucidate many points in connection with the life-history of the polymorph, and to place the polynuclear count on a scientific basis.

In order to illustrate the procedure, let us take a normal rabbit with a count of

I.	II.	III.	IV.	V.
12	34	38	8	8

which gives a weighted mean of 2·66. Most of the cells, it will be observed, lie in the central groups II. and III.

Fifty milligrammes of dried thyroid were extracted with saline, and the whole of the extract injected subcutaneously. The state of the count on successive days following the injection is shown in the following table:—

Day.	I.	II.	III.	IV.	V.
1	46	20	20	7	7
2	32	36	22	6	4
3	28	44	18	7	8
5	18	38	26	10	8
11	12	24	36	18	10
16	11	32	34	10	13
21	11	34	37	9	9

It will be seen that the changes in the count occur in a well-marked order. First, on the day after the injection the number of cells in Class I. is greatly increased, having risen from 12 to 46. The next stage is marked by a fall in the numbers in Class I. and an increase in the numbers in Class II. The numbers in Class I. become progressively less, and at the same time

the numbers increase first in Class II., and later in Class III., at which time there is an accompanying fall in Class II. Increase now occurs in Class IV. This is followed by a slight increase in Class V., after which the whole count reverts to normal.

In order to make the changes visible at a glance, the changes may be represented graphically as in fig. 5. Here the ordinates represent the numbers of cells in

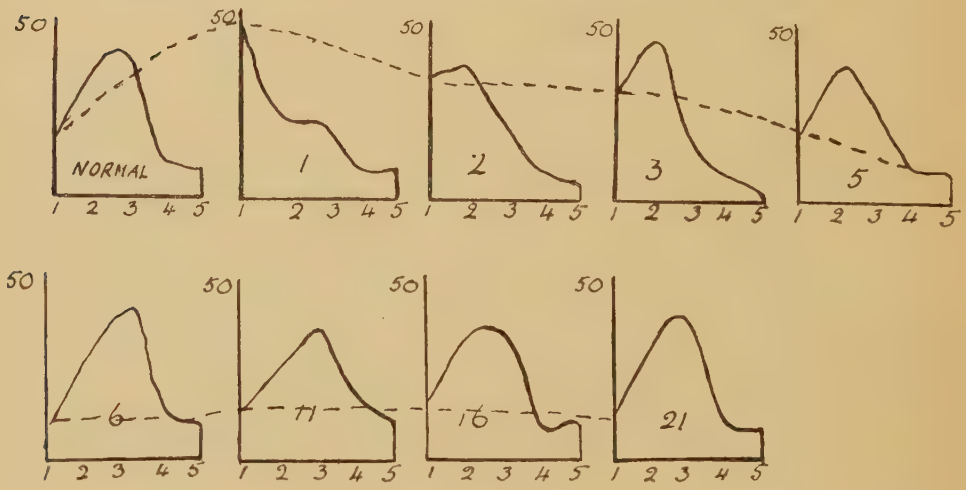

FIG. 5.

each class, the classes being marked off along the abscissa.

Three points should be noted particularly.

(1) The first effect of the injection is to produce a great increase in the number of polymorphs with simple nuclei. This increase is absolute as well as relative, for it is accompanied by a polymorph leucocytosis, as may easily be found by carrying out a polymorph count. The reaction occurs very quickly [1] and passes off rapidly, for

[1] With 50 milligrammes of thyroid it occurs within about four hours. The maximum numbers in Class I. occur about twelve hours after the injection.

within a day the numbers in Class I. show the fall which succeeds the first rise.

(2) The rise in class in Class I. is followed by a rise in Class II., and this by subsequent rises in Classes III., IV., and V. After the numbers in Class V. have attained their maximum, the count reverts to normal. Each successive rise is a little smaller than the one immediately before it, so that the final maximum in Class V. is produced by the addition of only a few cells.

(3) The first day after the injection there is a distinct maximum in Class I.; the maximum in Class II. takes place on the third day, that in Class III. on the sixth day, that in Class IV. about the twelfth day, and that in Class V. about eighteen days after the first deflection. The count reverts to normal after about three weeks.

These changes are typical of the effect of an injection of thyroid extract, although they may vary in extent according to the dose administered, as well as because of other more obscure factors. We are now left to explain them, and to do so we employ the following reasoning.

The relative increase in the numbers in Class I. which marks the beginning of the disturbance is accompanied by a polymorph leucocytosis. This means that a greater number of cells than normally have entered the blood-stream, and we assume that they enter from the blood-forming areas, principally from the bone marrow.

Let us suppose for a moment that these cells of Class I. do not develop in the blood-stream [1] into cells of Class II.,

[1] The reader should bear in mind that although the polynuclear count is made from the examination of cells drawn in a sample from the blood-stream, the polymorph population of the blood-vessels is only a fraction of the whole population of circulating polymorphs in the body. These cells abound in the extra-vascular spaces, from which they are continually entering the blood-stream, and into which they are continually passing from the vessels. When we speak of "pro-

that the cells of Class II. do not develop into cells of Class III., and so on, but that a cell of any class cannot leave that class except by death. Then we may account for the increase in the numbers in Class I. by a liberation of cells of Class I. from the marrow, but we must account for the increase in the numbers in Class II. which occurs on the third day in the same way, and must assume that these too are liberated from the marrow as cells of Class II., but at a later date than the first liberation of the cells of Class I. Similarly we have to imagine that the marrow, about the sixth day, liberates cells of Class III., and that about the twelfth and eighteenth days respectively it liberates cells of Classes IV. and V.

This hypothesis is so clumsy that it could not be seriously upheld, for one would require some explanation of this supposed intermittent and selective activity of the marrow. In addition to this, however, it may be completely disposed of by the observation that the marrow does not contain cells of Classes III., IV., and V. to liberate, for polymorphs of classes higher than Class II. are not found in the blood-forming areas.

If we abandon, as we must, this explanation, we have to substitute the hypothesis that there is a progressive development from class to class in the blood-stream. The increase in Class I. is now explained by saying that as the immediate result of the injection the marrow liberates cells of Class I., together with perhaps a few cells of Class II., into the blood. Having liberated a number which depends, among other things, on the dose of thyroid administered, it does no more. The

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duction" of polymorphs we mean additions of these cells to the entire system, and not merely additions to the blood-stream in the usual sense; similarly "removal" implies that the cells are removed from the entire system, *e.g.* by death, and not that they leave the vessels to pass into the tissues, from whence they may again return.

cells of Class I. now develop into cells of Class II. ; this gives the fall in the numbers in Class I. and the increase in the numbers of Class II. The cells of Class II. next pass into Class III., and the numbers in this class increase accordingly, and so on. Finally, the cells of Class IV. pass into Class V. ; these rise to a maximum, and then fall in numbers, for they depart from the blood-stream by death.

In view of the fact that the rises in successive classes become less and less as the development proceeds, it is certain that departures by death take place from the classes earlier than Class V., although the departures become greater and greater as the higher classes are reached.

In order to make the matter quite clear we may illustrate it by a diagram.

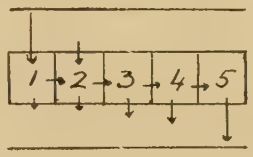


Fig. 6.

The diagrammatic representation is shown in fig. 6. Each of the five squares represents a class, beginning at Class I. on the left and ending with Class V. on the right. Above the row of squares is an area supposed to represent the bone marrow, below is an area into which the cells depart when they die. The polymorphs enter the blood-stream from the cell-producing areas as Class I., a few enter as cells of Class II. Hence we place arrows passing from the cell-producing area into these classes; the arrow entering Class I. is larger than that entering Class II. to indicate that the greater number of entries takes place into the former class. The cells of Class I. then develop into cells of Class II. ; this is represented by the horizontal arrow passing from Class I. to Class II. The development proceeds, as shown by the arrows, from class to class, the cells of Class II. become cells of Class III., these pass into Class IV., and finally into

Class V. From all the classes arrows pass downwards, increasing in magnitude from left to right; these indicate that the cells leave every class, but especially the later classes, by death.

From this position we may pass to the more difficult problem as to why there are normally more cells in Classes II. and III. than in the other classes. Although the reasoning which supplies us with the answer is rather involved,[1] the answer itself may be stated simply enough. The reason for there being comparatively few cells in Class I. is because the polymorphs which are liberated from the marrow do not remain long as cells of Class I., but pass rapidly on to become cells of Class II. In this state they remain for a longer time before they become cells of Class III., but in Class III. they remain longer still, for which reason Class III. contains more cells than Class II., just as Class II. contains more cells than Class I. It might be expected that that duration of stay in Class IV. would be longer still, and that in Class V. longest of all, but at this stage another factor enters. From these last two classes the now mature polymorphs are removed by death, and thus Classes IV. and V. contain comparatively small numbers.

It should be mentioned that the facts which lead up to this interpretation can be obtained from experiments with substances other than thyroid extract, for any

[1] The method by which the answer referred to is reached is one which depends on the numerical correspondence between (*a*) the number of cells in each of the polynuclear classes, and (*b*) the time taken for a maximum number of cells to appear in any particular class after a disturbance of the count. By this method we estimate the length of life of a polymorph in Class I. as one day, in Class II. as two days, in Class III. as four days, and in the higher classes as a little longer, provided that death does not take place. The whole length of life of a polymorph in the blood-stream is therefore about three weeks. For details, the reader must refer to original papers.

substances which will stimulate the cell-forming areas and which thus disturbs the steady state of the count will give the same results. From the injection of colchicine, nucleic acid, or trypsin, each of which causes a polymorph leucocytosis and a deflection of the polynuclear count, conclusions identical with those arrived at from the consideration of the effect of thyroid are obtained. The most recent work on the subject concerns the effect of exposure to X-rays (Kennedy and Grover); in this case too there is a deflection of the count and a polymorph leucocytosis, and from the state of the count from day to day as recovery proceeds the life-history of the polymorph can be worked out as identical with that described above.

The mechanism can be illustrated by a simple and useful analogy. We may consider, instead of the population of the blood-stream, the population of an island, and we may divide the people contained therein into classes according to their age. The first class we might take as the class of infants, the second class might be that of the children, the third class that of men up to the age of maturity, the fourth class one of men retired from work, and the last class that of people in the stage of extreme senility. Any individual must start life in Class I.; he progressed to Classes II., III., and IV. in orderly progression; and finally enters Class V., provided that he survives so long. He can never go back from Class II. to Class I., or from another class to the one before it; he must proceed from class to class, until at last he is inevitably overtaken by death, perhaps in Class V., perhaps in an earlier class. In this way the individual passes from class to class in sequence just as the polymorph does as it matures. Just as the cells may die and be removed from any class, so may the individual; he may die in infancy, in

childhood, in manhood, or in the later stages of life. In both cases, generally speaking, the chances of death increase with age. Finally, in the case of the population of the island, there are comparatively few infants, for an individual remains an infant for one year only ; he rapidly passes out of this class, just as does the recently liberated and immature cell. The contents of Class II., that of the children, will be greater, for a person remains a child for some fourteen years ; the contents of Class III. will be the greatest of all, for the individual remains a man for a long time. As in the case of the polymorph, the final Classes IV. and V., in which the individuals are old and senile respectively, will contain small numbers once more, for whenever an individual, cell or man, enters one of these classes the length of time during which he can hope to remain is limited by the limits of his life.

The essential point to grasp is that all the departures from Classes IV. and V. are compensated for by incoming individuals in Class I. ; the total number of the population thus remains constant, although there are continual additions and removals. This is equally true for a population of people or a population of cells, provided that the normal steady state is not disturbed.

What, then, do the figures of the polynuclear count tell us? They tell us whenever this normal steady state becomes altered, whether by addition of an unusually larger number of young cells, by the removal of an unusually large number of mature cells, or by some interference with the normal development.

In order to make this clear, consider a few examples.

Example 1.—Into a rabbit with an initial polynuclear count of

I.	II.	III.	IV.	V.
20	24	36	18	2

an injection of 10 c.c. of 1 per cent. trypsin was given subcutaneously. The initial polymorph leucocyte count of this animal, as determined by a total white-cell count and a differential count, was 4100 polymorphs per cubic millimetre.

Two hours after the injection there was a very marked polymorph leucocytosis, the count rising to 14,300 cells per cubic millimetre. On the basis of this increase it seems reasonable to assume that cells have been added to the blood-stream, but we cannot say from the polymorph count alone whether these cells are young cells or cells which are mature.

Taking the polynuclear count two hours after the injection, we find the figures :

I.	II.	III.	IV.	V.
58	28	10	4	0

It is now easy to discover the classes to which additions have been made, and to do so we proceed as follows :--

The ratio of the initial polymorph count to the count two hours after the injection is 14,300/4100, or very nearly 3·5. Multiply every figure of the polynuclear count as obtained after the injection by this figure, and we get

I.	II.	III.	IV.	V.
203	98	35	14	0

Now subtract the figures of the original polynuclear count. The result is

I.	II.	III.	IV.	V.
183	74	—1	—4	—2

These figures give us the additions and removals from the five classes. To Class I. there has been added from the bone marrow or other cell-producing tissues, polymorphs in the proportion of 183 of Class I. and 74 of

Class II. To Class III. there have been no additions, no doubt for the simple reason that the marrow does not contain cells of this class to add. There have been no additions to Classes IV. and V., but, on the contrary, a few removals. These removals are probably not significant, and could be made to disappear by taking into account the experimental error.

The injection has therefore brought about the deflection of the polynuclear count by a stimulation of the bone marrow and other cell-producing organs, with the result that immature cells of Class I. are added to the blood-stream in large numbers. With them, as very often happens, a number of cells of Class II. are added also.

This type of deflection, due to marrow stimulation pure and simple, is found when such drugs as thyroxin, nucleic acid, or colchicine are injected subcutaneously or intravenously.

Example 2.—A rabbit, rendered anæmic by frequent bleedings, showed on one day a polymorph count of 4500 per cubic millimetre, and a polynuclear count of

I.	II.	III.	IV.	V.
19	28	24	20	9

On the day after one of the bleedings, the polymorph count rose to 9000 per cubic millimetre. The question arises as to where the additional polymorphs came from, and is at once answered by an inspection of the polynuclear count. The figures were found to be

I.	II.	III.	IV.	V.
18	29	23	21	8

Multiplying by 9000/4500 or 2, and subtracting the original count, we get the additions to the various classes in the proportions

I.	II.	III.	IV.	V.
17	30	24	23	8

But these figures are those of a normal population, and almost identical with the figures for the polynuclear count on the day before the leucocytosis took place. The leucocytosis is therefore not caused by a production of young cells from the marrow, but by the entry into the circulating blood of a normal population of cells, probably from the tissue spaces.

It is of interest to observe that this example illustrates very well what occurs in "digestion leucocytosis" in man. It has frequently been claimed that the polymorphs which appear in the blood-stream after a heavy meal are derived from the marrow. This, however, is not the case, for there is no simultaneous deflection in the polynuclear count; the cells are those of a perfectly normal population, and are added to the circulation from reserves in the tissues.

Example 3.—A rabbit with a polymorph count of 5200 per cubic millimetre and a polynuclear count of

I.	II.	III.	IV.	V.
28	42	22	8	0

received an exposure to X-rays. On the day following the exposure the polymorph count had risen to 10,000 per cubic millimetre, and the polynuclear count was found to be

I.	II.	III.	IV.	V.
78	16	6	0	0

Multiplying by 10,000/5100 or by 2 very nearly, and subtracting the original count, we get the additions as

I.	II.	III.	IV.	V.
128	—10	—10	—8	0

The change in the count is thus due to the addition of a large number of immature cells from the marrow, with the simultaneous removal of cells from Classes II., III.,

and IV. Cells are doubtless removed also from Class I., but the additions to this class obscure these removals.

Example 4.—A rabbit with a normal polymorph count of 10,000 per cubic millimetre and a polynuclear count of

I.	II.	III.	IV.	V.
20	29	40	9	2

was infected experimentally with tuberculosis. After twenty days there was a well-marked polymorph leucopenia, with a count of only 5000 per cubic millimetre, and a polynuclear count of

I.	II.	III.	IV.	V.
49	40	11	0	0

Multiplying by 5000/10,000, or dividing by 2, and subtracting the original count, we get the additions and removals from the various classes as

I.	II.	III.	IV.	V.
5	—9	—35	—9	—2

The effect of the experimental tuberculosis is therefore to remove the cells of the higher classes, thus rendering the count "left-handed," but at the same time to render the marrow active. This is shown by the fact that not only are cells not removed proportionately from Class I., but there are actual additions to the numbers in this class. We have, therefore, the apparently paradoxical occurrence of a leucopenia with an activity of the bone marrow; strange though it may appear, this condition is the rule in such conditions as tuberculosis.

These examples serve to show in some measure the uses to which the polynuclear count may be put. Either taken alone or in conjunction with the total polymorph count, it yields the most valuable information regarding the extent of marrow activity on the one hand, and of white-cell destruction on the other. Fortunately it is

remarkably constant in health, and at the same time readily susceptible to alteration in abnormal conditions; its value for the elucidation of physiological problems connected with the white-cell population must already be apparent, but its use is even greater in the diagnosis and prognosis of many pathological conditions, to the consideration of which we now pass.

CHAPTER III.

THE POLYNUCLEAR COUNT IN THE INFECTIVE STATE.

As we have seen in Chapter II., under experimental conditions the count is deflected by a variety of toxic agents, and the left-handed drift depends upon the marrow reaction. In the infective state the same increase in Classes I. and II. and consequent diminution in the cells of Classes III., IV., and V. occurs. This left-handed deflection of the count is present at the inception of all infective conditions, and continues throughout the disease, the count varying with exacerbations and remissions until immunity is finally established, when the percentages of the cells of the various classes assume their normal proportion. The deflection is present in all cases of infection, the exanthemata, pyogenic infections, local and general, and in diseases caused by the filterable viruses.

Examples of this are given in the table on the following page.

This dislocation occurs whether the condition is characterised by a leucopenia or a leucocytosis. For example :—

Typhoid Fever—

Total leucocytes, 2300 per c.mm. Polymorphs, 59 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	54	31	15	0	0

Measles—

Total leucocytes, 800 per c.mm. Polymorphs, 71 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	72	24	4	0	0

	I.	II.	III.	IV.	V.
Typhoid fever	54	31	15	0	0
Scarlet fever	44	43	13	0	0
Measles	45	39	16	0	0
Erysipelas	45	40	14	1	0
Diphtheria	41	39	17	3	0
Rubella	30	35	32	3	0
Varicella	33	35	28	4	0
Pertussis	34	39	27	0	0
Puerperal sepsis	42	37	21	0	0
Gonorrhœa	20	31	40	8	1
Acute anterior poliomyelitis	39	27	27	7	0
Dental sepsis	26	38	30	6	0
Cerebrospinal fever	40	45	14	1	0
Streptococcal septicæmia	64	36	0	0	0
Staphylococcal osteomyelitis	47	41	12	0	0
Chronic nasal catarrh	28	31	37	4	0
Lobar pneumonia	57	26	15	2	0
Chronic bronchitis	25	45	23	7	0

Pulmonary Tuberculosis—

Total leucocytes, 4200 per c.mm. Polymorphs, 54 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	34	44	18	4	0

As we have pointed out in the previous chapter, a leucopenia does not necessarily mean aplasia of the leucopoietic tissues; actually, in these cases, there is marrow activity.

Examples of the deflection of the count with a leucocytosis are:—

Cerebrospinal Fever—

Total leucocytes, 26,800 per c.mm. Polymorphs, 74 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	56	27	14	3	0

Acute Lobar Pneumonia—

Total leucocytes, 23,400 per c.mm. Polymorphs, 89 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	62	32	5	1	0

Streptococcal Septicæmia—

Total leucocytes, 26,000 per c.mm. Polymorphs, 79 per cent.

	I.	II.	III.	IV.	V.
Polynuclear count	34	43	21	2	0

We have not found any exception, experimental or clinical, to the statement made above, viz. that a left-hand deflection of the count occurs in all cases of infection.

Lest we be misunderstood, it is necessary at this juncture to mention a disease which is generally supposed to be caused by an intestinal infection of hæmolytic organisms. In pernicious anæmia the polynuclear count frequently shows an increase in Classes IV. and V. and the count is actually right-handed. The following case is a typical example:—

F., Male, aged 36 years.

18/3/1926.

Hæmoglobin	27 per cent.
Erythrocytes	1,300,000 per c.mm.
Leucocytes	3000 per c.mm.
Colour index	1·0.

Differential Count—

Polymorphs	48 per cent.
Lymphocytes	34 ,,
Monocytes	10 ,,
Myelocytes	8 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
10	15	38	24	13

Megalocytes and megaloblasts were very numerous. Van den Bergh's indirect reaction was positive, the urine contained urobilinogen, there was achlorhydria, and retinal hæmorrhages were present in both fundi. W. R., negative. On 5/4/1926 the patient developed acute lobar pneumonia, and on 8/4/1926 his leucocyte count was

Total leucocytes . 28,600 per c.mm.

Differential Count—

Polymorphs	87 per cent.
Lymphocytes	12 ,,
Monocytes	0·5 ,,
Myelocytes	0·5 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
36	38	21	5	0

The patient died on 11/4/1926. This case is interesting in many respects. The count at first was markedly right-handed in spite of the fact that there were 8 per cent. of myelocytes in the blood-films. The advent of acute lobar pneumonia heralded a revolution in the blood picture. The leucocytosis and increase in polymorphs which occurred prove very definitely that the leucopoietic tissues could still react to an added infection. This is frequently seen in cases of pernicious anæmia with infections by pyogenic organisms, but some cases do not show any reaction at all to infection. Although

this case showed a marrow reaction to a massive pneumococcal infection the polynuclear count is not dislocated to the same extent as it would be in normal individuals. Involvement of all three lobes of the right lung would ordinarily give a polynuclear picture approximating to

I.	II.	III.	IV.	V.
57	26	15	2	0

and the nuclei of the polymorphs would be densely stained. In this case the polynuclear count was as above, and the nuclei of the polymorphs were very poor in chromatin.

If the etiological factor proves to be a microbic infection, then pernicious anæmia will be the outstanding exception to the general statement made above. Further reference to the disease will be made in Chapter IV.

As we have mentioned, a leucopenia is consistent with marrow activity. The reverse of this condition is true, viz. a leucocytosis does not always mean marrow reaction. The case of the bled rabbit quoted in Chapter II. has its parallel in man.

A. H., Male, aged 31 years.

3/4/1927. History of intestinal hæmorrhage for the past three weeks, had been in bed for four days. The blood showed the following condition:—

Hæmoglobin	.	55 per cent.
Erythrocytes	.	3,900,000 per c.mm.
Leucocytes	.	7500 „
Colour index	.	·7.

Differential Count—

Polymorphs	.	64 per cent.	=	4800 per c.mm.
Lymphocytes	.	30 „	=	2250 „
Monocytes	.	3 „	=	225 „
Eosinophils	.	2 „	=	150 „
Mast cells	.	1 „	=	75 „

Polynuclear Count—

I.	II.	III.	IV.	V.
16	31	40	11	2

Two days later (5/4/1927) he got out of bed and dressed, walked to a taxi, drove three miles to the infirmary, and walked from the taxi to the X-ray room. The total distance walked was 100 yards. His count then was:

Total leucocytes	12,600 per c.mm.	
Polymorphs	61 per cent.	=7686 per c.mm.
Lymphocytes	33 ,,	=4158 ,,
Monocytes	4·5 ,,	= 567 ,,
Eosinophils	1·0 ,,	= 126 ,,
Mast cells	0·5 ,,	= 63 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
13	33	37	13	4

At operation a small duodenal ulcer was found.

Using the same methods as in the case of the rabbit, we find that the additions to each class have been

I.	II.	III.	IV.	V.
5	21	19	10	4

As the marrow does not contain cells of Classes III., IV., and V., the increase in the total leucocytes must have been due to mobilisation from the tissues and not due to marrow activity.

These counts are interesting because they show only a slight left-handed deflection. The duodenal ulcer was not larger than the size of a large pin-head.

That there is a close connection between the general clinical condition and the polynuclear picture is shown by the fact that if the case becomes clinically worse, the left-handed " drift " becomes greater. But if the

case tends towards recovery, the numbers in Classes I. and II. diminish, those in Classes III., IV., and V. increase until, when the patient is free from toxæmia, the picture resumes its normal appearance. This is seen in the following table :—

Female, æt. 12 *Years.*

Date.	I.	II.	III.	IV.	V.	Remarks.
July 1, 1914	4	23	49	20	4	In good health.
„ 22, „	25	39	30	5	1	Acute tonsillitis on 12th July 1914.
Aug. 5, „	11	41	36	10	2	
„ 17, „	13	37	39	10	1	
Sept. 20, „	4	22	47	23	4	Recovered.
Oct. 29, „	9	27	49	13	2	Nasal catarrh.
Nov. 15, „	8	30	39	21	2	
„ 29, „	4	23	48	22	3	Recovered.

On 1st July a film was taken after a clinical and X-ray examination to exclude infection. The count confirmed the opinion that the child was in normal health. On the 12th she developed an attack of tonsillitis. The film of the 22nd shows the count to be still dislocated to the left. On 20th September it had returned to normal. On 27th October she developed a slight nasal catarrh, but she was not confined to the house. There was a slight dislocation to the left. On the 29th November the count had assumed its normal appearance.

It is interesting to note how the blood in health, taken at different times, works out at practically the same figure in the various classes. The sensitiveness of the polynuclear count to toxæmia is also seen. There is one other point about the case. The chart indicates that the girl's health figures were 26 or 27 in the left-handed columns. Having seen it firstly in her usual

health on 1st July, and secondly after recovery from an attack of tonsillitis on 20th September, it was possible to deduce, from the blood of 29th October, that she had some slight source of toxæmia, although her count had not gone past what is generally taken as the limit of the normal standard, namely, 40 in columns I. and II. This point should be carefully observed, for, as reference to Appendix II. will show, there is considerable variation in the normal polynuclear count. A person who happened to have quite a normal count with the following figures,

I.	II.	III.	IV.	V.
3	23	49	21	4

might, as the result of a slight toxæmia, develop a count of

I.	II.	III.	IV.	V.
17	21	48	10	4

which, taken as it stands, is not abnormal, but which, taken together with the previous normal count for the individual, is a very definite evidence of toxæmia. Were the count of this individual to change to

I.	II.	III.	IV.	V.
19	26	46	9	0

the change would indicate a considerable amount of toxæmia, while this last count would have but little significance if found in a person whose normal count happened to be

I.	II.	III.	IV.	V.
12	29	46	12	1

It is important to bear these possibilities in mind, especially as in clinical work one rarely has the opportunity of making careful determinations of the normal counts of each patient.

Other examples of the return of the polynuclear picture to normal after infections are the following :—

A. G. Pulmonary Tuberculosis.

Date.	I.	II.	III.	IV.	V.
Nov. 19, 1913	43	36	16	5	0
Feb. 15, 1914	26	37	31	6	0
May 28, „	26	28	39	6	1
Aug. 29, „	11	29	43	16	1

On admission this was an early left apical infection with a considerable amount of general bronchitis ; tubercle bacilli were occasionally present in the sputum in very scanty numbers. In August 1914 he was doing six hours hard manual work daily. There was no cough nor sputum, temperature and pulse rate were normal, and the chest was free from physical signs of active disease.

P. C. Diphtheria.

On 20th December 1914, P. C., a child aged nine years, had a small patch of membrane the size of a threepenny piece on the right tonsil caused by *B. diphtheriæ*. She had been ill for twelve hours. Her count on the morning of that date was

I.	II.	III.	IV.	V.
26	39	32	3	0

Ten thousand units of antitoxin were administered, and on 6th January 1915, eighteen days afterwards, her count had returned to normal :

I.	II.	III.	IV.	V.
5	31	47	16	1

This is the most rapid recovery of the count in infection in our experience, and corresponds closely with the results

of thyroid injection in rabbits. The 6th January count shows that the amount of antitoxin administered on 20th December was sufficient.

N. W. Infective Jaundice.

This case was taken ill five days previous to the first count being taken. She commenced with a temperature of 101° F., and on the day her count was done her temperature was 99° F., Van den Bergh's reaction, direct negative, indirect positive.

Polynuclear Count.

Date.	I.	II.	III.	IV.	V.
29/10/26	46	46	8	—	—
5/11/26	22	56	22	—	—
12/11/26	18	40	34	8	—
19/11/26	18	32	42	8	0
8/12/26	12	30	44	12	2
15/12/26	10	28	44	16	2

Two Cases of Removal of Infected Tonsils and Adenoids.

	Date.	I.	II.	III.	IV.	V.
Case 1.	23/1/24	25	36	36	3	0
	Operation.					
	14/3/24	8	28	44	18	2
Case 2.	23/1/24	22	42	30	6	0
	Operation.					
	14/3/24	4	26	42	26	2

Staphylococcal Infection.

N. McC. had an infection of the patellar bursa, lymphangitis and lymphadenitis of the glands in

Scarpa's triangle. The bursa was opened on 13/10/26. Her counts were as follows :—

Date.	I.	II.	III.	IV.	V.
13/10/26	46	32	21	1	0
21/10/26	33	41	25	1	0
3/11/26	18	42	32	8	0

On 4/11/26 the patient had a rigor, temperature of 100° F., and acute tonsillitis. The effect on the count is seen :

Date.	I.	II.	III.	IV.	V.
5/11/26	55	30	15	0	0
12/11/26	40	46	12	2	0
19/11/26	22	42	32	4	0
3/12/26	12	38	38	12	0
5/ 1/27	10	22	46	18	4

These examples illustrate the behaviour of the count in the presence of infection and recovery from infection. The time taken for the count to return to normal is longer in disease than the time required under experimental conditions. In the latter we are dealing with a single dose of a toxic agent which is incapable of multiplying and adding to its toxicity. In the infective state many more factors are present—the size of the infection, the toxicity of the organism and its products, its power of continued growth, the amount of toxins absorbed and their gradual neutralisation by antibodies. So long as any toxin remains unneutralised, the count will remain left-handed.

The count is not prophetic. It is an index of marrow reaction, and the amount of dislocation depends upon the severity of the toxæmia which is present at the time the blood is taken. Clinically this is seen by comparison of the counts in severe general infections as typhoid,

scarlet fever, cerebrospinal fever, pneumonia, etc., with those in more local infections such as gonorrhœa, dental sepsis, etc. It is reasonable to suppose that, given two lesions caused by the same organism, there will be more absorption of toxins from the larger, and the polynuclear count should, on that hypothesis, be more dislocated than in the smaller lesion. This is, in fact, what is found. Tuberculosis of the larynx, with a small absorptive area, shows a much less dislocated picture than a lesion with a large absorptive area such as tuberculous pleurisy. For example:

Early Laryngeal Tuberculosis.

Case.	I.	II.	III.	IV.	V.
1	17	37	31	14	1
2	16	36	38	9	1

Tuberculous Pleurisy.

Case.	I.	II.	III.	IV.	V.
1	46	39	13	2	0
2	67	25	8	0	0

An early case of pulmonary tuberculosis with a small area of activity shows a less marked dislocation than one more advanced, and this again will present a smaller change than the far advanced case.

As the active areas decrease, the polynuclear count assumes a more right-handed appearance until, when all activity has ceased, it returns to normal figures.

Early Pulmonary Tuberculosis.

Case.	I.	II.	III.	IV.	V.
1	20	44	29	6	1
2	25	40	33	2	0
3	25	25	40	9	1
4	23	31	37	8	1

Moderately Advanced Cases.

Case.	I.	II.	III.	IV.	V.
1	30	40	25	4	1
2	37	36	23	3	1
3	32	36	27	5	0
4	32	41	23	4	0

Advanced Cases.

Case.	I.	II.	III.	IV.	V.
1	45	33	18	4	0
2	46	40	11	3	0
3	42	44	14	0	0
4	54	40	6	0	0

Arrested Cases.

Case.	I.	II.	III.	IV.	V.
1	8	21	47	22	2
2	12	29	46	12	1
3	11	25	47	16	1
4	12	31	40	14	3

And cases of tuberculosis, which already present left-handed counts, show still further dislocation if any increase in the toxæmia takes place. This is seen in the following cases of pulmonary tuberculosis:—

Case 1.—*Pulmonary Tuberculosis, progressing favourably. Contracted slight Tonsillitis on* 27*th November* 1914.

Date.	I.	II.	III.	IV.	V.
29/10/14	27	38	34	1	0
29/11/14	32	46	21	1	0

Case 2.—*Pleurisy on* 14/3/14.

Date.	I.	II.	III.	IV.	V.
8/3/14	41	42	16	1	0
15/3/14	61	34	5	0	0

Case 3.—Pulmonary Tuberculosis. The count in this case was further dislocated as the result of auto-intoxication due to excessive walking.

Date.	I.	II.	III.	IV.	V.
29/10/14	33	45	20	2	0
29/11/14	49	42	9	0	0

On 27/11/14 the patient walked two miles contrary to instructions. The effect on the polynuclear count is seen.

Joint tuberculosis provides further proof.

If a tuberculous joint be fixed, there is lessened absorption from it, and the polynuclear count, previously dislocated to the left, moves to the right. But if the joint be moved before it is healed, there is a sharp increase in the number of cells in Classes I. and II.

Tuberculosis of Right Hip-joint (Mr. M'Crae Aitken's Case).

Date.	I.	II.	III.	IV.	V.
18/8/13	26	43	26	5	0
Joint fixed on 19/8/13.					
28/9/13	9	42	36	13	0
Movement on 30/10/13.					
2/11/13	16	39	41	4	0
9/11/13	33	42	20	5	0

The joint was fixed on 19/8/13, and the effect on the count was very marked in a month. The case continued to improve until movement was commenced on 30/10/13. Three days afterwards increased absorption had already shown itself, and in a week the left-handed appearance of the count was pronounced.

Experimental tuberculosis in rabbits has been studied, and the hæmatological findings bear out all that has been said in reference to human tuberculosis.

We must next consider the significance of these changes in the polynuclear picture. Since Arneth's first work in 1904, a considerable volume of literature has been published on the subject, some of it teeming with arrant nonsense. Three statements that have appeared in authoritative text-books on hæmatology during the past few years are typical examples. They are:

1. The cells with 3, 4, and 5 lobes are better phagocytes than those with 1 and 2 lobes.
2. The polymorphs with 3 or more lobes are more resistent than the immature form with 1 and 2.
3. A high percentage of cells in Classes I. and II. is unfavourable in prognosis.

We know from experimental and clinical evidence that the one- and two-lobed cells are younger than the III., IV., and V. classes, and their presence in numbers in the blood-stream is evidence of marrow activity. There is not the slightest evidence that the cells of the higher classes have more phagocytic power than the younger cells. Working on this subject independently we have come to the same conclusion, which may be exemplified as follows:—

	I.	II.	III.	IV. and V.
Staphylococcus aureus—				
Mean number of cocci per cell . .	2·1	2·8	2·1	2·5
M. catarrhalis—				
Mean number of cocci per cell . .	2·8	2·9	2·6	3·0

B. coli—				
Mean number of bacilli per cell . .	2·8	2·4	2·5	3·0
B. tuberculosis—				
Mean number of bacilli per cell . .	3·1	3·4	2·9	3·6
The average of a large number of counts is	2·5	2·7	2·6	2·7

These figures concern phagocytosis *in vitro*, but precisely the same results are obtained when ingestion of bacteria is allowed to proceed in the peritoneal cavity of the rabbit. In this case the polymorphs have to pass into the cavity from the blood-stream; the cells of one class diapedese as readily as those of the other classes, so that the polynuclear count carried out in the cells of the exudate is identical with that of the cells of the blood-stream. Once in the peritoneal cavity, the cells ingest the bacteria to the same extent, irrespective of their class. If the findings had shown that the higher classes were the more phagocytic an explanation of the position would have to be found. We should have, in the very states where phagocytic activity is most necessary, a diminution, often amounting to almost complete loss, of the very types of polymorph that were supposed to be specially endowed with that power. It is certain that nuclear lobulation has no bearing on phagocytic power, and the work, in those diseases where phagocytosis plays an important rôle, can be delegated with safety to the younger generations that are called into action. The older members, already tottering on the brink of the grave, far from being more resistent, are soon vanquished and leave the polymorph population by death.

The third statement, that a high percentage of cells

in Classes I. and II. indicates an unfavourable prognosis, is only partially true. If the count remains markedly left-handed, then the fact will have to be duly considered with other clinical data when arriving at a prognosis. Acute massive infections such as lobar pneumonia and cerebrospinal fever frequently have counts of 85 to 95 in Classes I. and II., and recover. Some have sought an explanation of the phenomena in that indefinite term "resistance of the patient," and have looked upon a markedly deflected count as an index of "lack of resistance." Upon what evidence this idea germinated we do not know, but a glance at the examples in disease and the experimental counts should dispel this illusion until such time as the meaning of "resistance" is known.

The true explanation of the alteration of the count in infective states is, we believe, the production of immunity.

Infection takes place and the marrow responds by flooding the blood-stream with young polymorphs, the numbers depending upon the severity of the infection. The nuclear activity of the marrow cells and the pouring into the circulation of large amounts of chromatin is probably associated with the elaboration of such enzymes as are necessary to combat the invasion.

Metchnikoff first suggested this theory when it had been shown that phagocytosis was only one of many factors in recovery from disease. He contended that the enzymes which performed intracellular digestive functions were the same as the enzymes of the blood-stream, and that the latter were of leucocytic origin. Against this Moxter, Pettersen, Schattenforh, Zinsser, and others have brought experimental findings based on extraction processes. We need not lay too much

stress on their results, because it is difficult to see how the links in the chain chromatin-prezymogen-zymogen are accounted for in the end products of simple extraction processes. The problem increases in complexity when we remember that the final enzyme is often specific, as can be shown in some infections, or by the parenteral introduction of certain foreign proteins.

Some objection may be raised to this theory on the ground that in certain diseases there is a leucopenia. A leucopenia does not necessarily mean marrow aplasia, but is consistent with activity. It may be that in these diseases destruction is greater than production, or the toxins of the causative agent bring about a reaction of only slight degree in the marrow, but more especially in certain fixed tissue cells, upon which devolves the duty of antibody production.

The Count in Diagnosis, Prognosis, and Treatment.—From what has gone before, the utility of the count as an adjunct to the clinical methods in diagnosis will be obvious to the reader, and to repeat and enlarge on specific cases would be redundant. Its value in diagnosis lies in the fact that if the count is dislocated to the left the patient is in the infective state. Having that point definitely settled is of great assistance to the clinician, and the search for the focus of infection undertaken with the confidence that one exists.

Hosts of examples could be quoted where the count has been valuable in the diagnosis of obscure infections—dental sepsis, cholecystitis, infections of the accessory sinuses, intracranial abscess, subphrenic abscess, and secondary anæmias, to mention only a few.

In the diagnosis of early tubercle the count has a definite niche. The persistent left-handed count with a leucopenia and an increase in the monocytes is always a suspicious picture.

There are conditions, too, where the elimination of infection is important. Let us take two very common cases. The first a case of pulmonary tuberculosis without sputum or pyrexia, but with dyspnœa on exertion and physical signs of local pulmonary fibrosis.

"Is this patient still suffering from tuberculosis, or are his symptoms due to mechanical factors consequent on healing?" That question can be answered in a few minutes by examination of the polynuclear count.

Fig. 7.

The second example would appear a protest against the attempt to render the population edentulous. It is also an example of the dentist's skill.

The patient, Mrs. F., was sent by her dental surgeon, Mr. F. D. Lamb, in order to ascertain the presence or absence of toxic absorption. She had 27 teeth present, of which 23 were pulpless, 17 of which were crowned. A radiograph showed rarefied areas $\frac{2\,|}{21\,|\,12}$. An acute abscess in the lower incisor area occurred in 1917, eight years ago; this was treated.

On 12th October 1923 her blood count was as follows:—

Hæmoglobin	.	98 per cent.
Erythrocytes	.	4,800,000 per c.mm.
Leucocytes .	.	6800 ,,

Differential Count—

Polymorphs .	.	63·5 per cent.
Lymphocytes	.	27·5 ,,
Monocytes .	.	5·5 ,,
Eosinophils .	.	3·5 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
6	31	47	14	2

This showed definitely that no absorption was taking place. The count was done in ignorance of the condition. The patient was, and still is, in the best of health.

On 14th September 1926 her reds, whites, and differential count were almost identical with that of three years previous.

Her polynuclear count was

I.	II.	III.	IV.	V.
6	27	45	20	2

and confirmed the opinion of her dentist that no lame teeth were present.

In prognosis the value of counts repeated at intervals, according to the acuteness or chronicity of the disease, is apparent. The unfavourable case has an increasing left-handed drift; the hopeful, a count that shows progressive diminution of cells in Classes I. and II. It is obvious that a single count cannot be of great help in prognosis beyond the fact that it is an index of the severity of the infection. It will not tell the observer whether the fight for supremacy is going to the host or the invader. Blood-films taken at later intervals will give the answer. In pulmonary tuberculosis the count, taken in conjunction with the clinical and X-ray evidence, completes the view and gives an insight into the case hitherto unobtainable. If a case comes under notice for the first time with a localised apical lesion, and the polynuclear count is markedly left-handed, more dislocated than experience tells us it should be with that type of lesion, X-ray examination invariably reveals much more widespread disease than can be detected by the stethoscope. The improving case

shows a gradual return of the count towards normal figures as the case mentioned on p. 40.

The stationary case has a stationary count:

Date.	I.	II.	III.	IV.	V.
17/1/14	48	37	15	0	0
30/1/14	42	42	16	0	0
14/2/14	46	38	14	2	0
8/3/14	42	41	16	1	0
21/3/14	53	32	15	0	0

Here the count is practically the same for months, the invaders and defenders have fought a drawn battle up to now, but it is obvious the stress of conflict must have an effect sooner or later. The state of equilibrium between the toxins absorbed and the marrow reaction cannot continue indefinitely. The defences may get the upper hand, the polynuclear count then becomes less dislocated, or the invaders may triumph, the count showing progressive increases in Classes I. and II., and death terminate the conflict. The same remarks apply to all cases of infection.

In estimating the results of treatment the count is invaluable, and the time has come when it should be used as the criterion of cure. Its utility in cases of focal sepsis and in treatment by vaccines and sera requires no further comment.

The vendors of infallible remedies for tuberculosis deny themselves the salutary lessons of the dead-house, but there is no reason why someone should not teach them the equally salutary lessons learned from a blood-film.

In concluding this chapter on the infective state, we must impress on the reader that the polynuclear count must not be used to replace any other method. Nothing can take the place of a careful clinical examination, the count merely completes the picture of the disease and is a pointer to the way the case is progressing.

CHAPTER IV.

THE MACROPOLYCYTE.

During the past twenty-five years isolated observations on the presence in the peripheral circulation of large cells having the appearance of megakaryocytes have been recorded by Bunting, Oelhafen, Di Guglielmo, Nageli, Pianese, Pittaluga, and others.

These cells have been called megakaryocytes or pleocaryocytes. To these terms we take exception, as they suggest a direct origin from the hæmocytoblast, an indiscriminate output of a primitive circulatory cell to which no function in the peripheral blood can be assigned.

The function of the megakaryocyte of the marrow is supposed to be the manufacture of thrombocytes. In addition to acting as the parent cell of platelets, Jordan suggests that, under conditions of intense hæmopoiesis, the cell functions as a hæmogenic giant cell. Nuclear segments detach themselves from the main body of the nucleus, hæmoglobin is developed round them, and they are extruded from the cytoplasm as erythroblasts.

Further, no reference has hitherto been made to the fact that the morphological characters of these large cells divide them into two groups which merge into each other. All the family resemble enlarged polymorphs, some members definitely so, some resemble the megakaryocyte of the bone-marrow, and others again are intermediate between the two extremes.

The term megakaryocyte or pleocaryocyte cannot be applied to the whole family, and for reasons which will be

apparent later, we have adopted the descriptive title of "Macropolycyte," suggested by Sir Humphry Rolleston.

The macropolycyte has been found in a variety of conditions, tuberculosis, cancer, the acute pyogenic infections, the exanthemata, pernicious anæmia, and anæmias due to ricin and repeated hæmorrhages, and in myelogenous leukæmia. It is also found in the rabbit after repeated hæmorrhages.

The normal polymorph measures from 10 to 12 microns in diameter, fig. 8. The cells to which we now refer are giants measuring from 14 to 21 microns in diameter.

They are of two distinct types. Macropolycytes of type I. are illustrated by figs. 9 to 16. The staining reactions of the nuclei and cytoplasm are identical with those of the other polymorphs in the blood-film. The nuclei are frequently hypersegmented—a marked contrast to the other polymorphs which show a large preponderance of cells with one- and two-lobed nuclei.

Macropolycytes of type II. are seen in figs. 17 to 21. These may be termed the megakaryocyte type as they approximate in size and nuclear conformation to the megakaryocyte of the marrow.

The nucleus is gnarled, may be of the simple horseshoe type showing protuberances, or may have 10 to 14 or more segments. The chromatin content of the nucleus is relatively poor in many, and the cytoplasm contains large granules which are not all oxyphil like the other polymorphs in the film, but mixed, some being oxyphil, others azurophil.

Intermediate between these two types are cells which resemble both. Fig. 24 is an example. The nuclear segments are separated by larger intervals than in the megakaryocyte type, the intervals being bridged over by fine chromatin filaments as in type I. The granules of the cytoplasm are almost all oxyphil, and in size

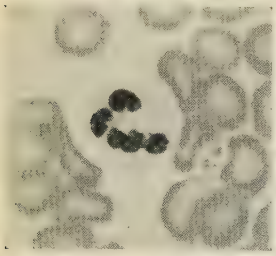


Fig. 8.

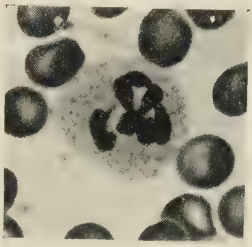


Fig. 9.

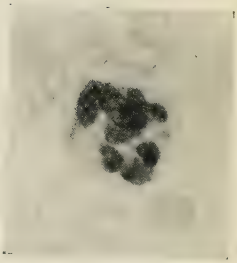


Fig. 10.

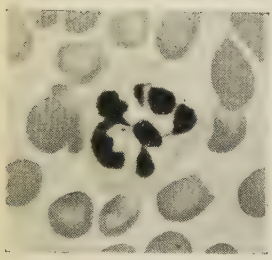


Fig. 11.

Fig. 12.

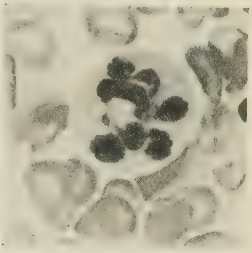


Fig. 13.

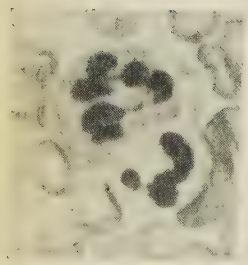


Fig. 14.

Fig. 15.

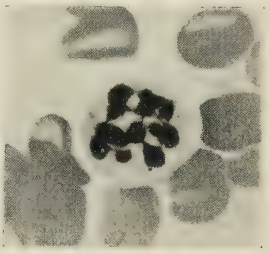


Fig. 16.

Fig. 8.—Normal polymorph. × 1000. Diameter, 11·5 μ.

Figs. 9 and 10.—Macropolycytes (Type I) from a case of staphylococcal osteomyelitis. × 1000. Diameters, 9, 18·5 μ. 10, 17 μ.

Fig. 11.—Macropolycyte (Type I) from a case of streptococcal septicæmia. × 1000. Diameter, 19·5 μ.

Figs. 12 and 13.—Macropolycytes (Type I) from a case of a mixed infection. *Staph. aureus* and *B. coli*. × 1000. Diameters, 12, 18 μ. 13, 17 μ.

Figs. 14 and 15.—Macropolycytes (Type I) from a case of streptococcal endocarditis. × 1000. Diameters, 14, 19 μ. 15, 16 μ.

Fig. 16.—Macropolycyte (Type I), pernicious anæmia. × 1000. Diameter, 16 μ.

approximate the normal polymorph rather than the large granules of the megakaryocyte type.

The following are examples of macropolycytes of type I. Figs. 9 and 10 are from a case of staphylococcal osteomyelitis :—

Total leucocytes, 18,000 per c.mm.
Polymorphs, 87 per cent. Myelocytes, 0·5 per cent.

Polynuclear Count—

I.	II.	III.	IV.	V.
41	43	15	1	0

Fig. 11 is from a case of streptococcal septicæmia :

Total leucocytes, 26,000 per c.mm.
Polymorphs, 79 per cent. Myelocytes, 0·5 per cent.

Polynuclear Count—

I.	II.	III.	IV.	V.
34	43	21	2	0

Figs. 12 and 13 are taken from blood-films in a case of a mixed terminal infection by *Staphylococcus aureus* and *Bacillus coli*:

Total leucocytes, 23,700 per c.mm.
Polymorphs, 88 per cent. Myelocytes, 1·0 per cent.

Polynuclear Count—

I.	II.	III.	IV.	V.
45	39	15	1	0

Figs. 14 and 15 are from a case of ulcerative endocarditis due to a streptococcal infection :

Total leucocytes, 16,000 per c.mm.
Polymorphs, 76 per cent.
Myelocytes were present in every blood-film.

Polynuclear Count—

I.	II.	III.	IV.	V.
39	34	27	0	0

These macropolycytes of type I. have hypersegmented nuclei, although in the polynuclear count cells with simple one- and two-lobed nuclei markedly predominate, as the foregoing counts show. It will be noticed too that in all the cases there is a polymorphic leucocytosis, and myelocytes are present in the peripheral blood, evidence of marrow stress.

Fig. 16 is an example of type I. in pernicious anæmia, and it will be seen to resemble the type I. macropolycyte found in other conditions.

The nucleus and cytoplasm have the same staining reactions as the other polymorphs in the blood-film.

Type II. or the megakaryocyte type is illustrated by figs. 17 to 21.

Now the question arises, " What are these cells ? "

The life-history of these abnormal polymorphs appears to be similar to the life-history of the normal cell.

There does not appear any doubt that these cells commence life in the blood-stream as simple nucleated types. The small cells in figs. 17 and 19 present the early megakaryocyte type, and from comparison with the normal life-history we must conclude that, as they grow older, their nuclei become hypersegmented. The macropolycyte of type I. commences its career in the same way, but, of course, is indistinguishable from the ordinary polymorph until the adult stage is reached. Their length of life we do not know, nor do we know their function, but we can hazard with some degree of confidence the cause of their production and probable genesis.

It has been suggested that these large hypersegmented polymorphs represent pathological ageing due to abnormal stimuli acting on the cell, a consecutive and intense hyperfunctioning of the granulocyte, together with delay or absence of the physiological mechanism

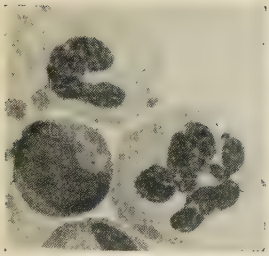


Fig. 17.

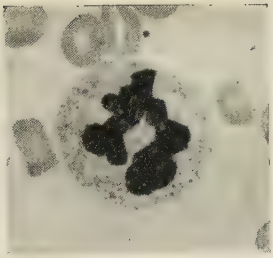


Fig. 18.

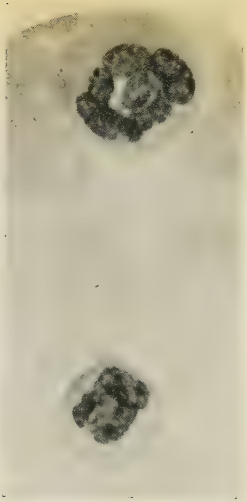


Fig. 19.

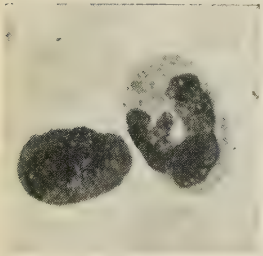


Fig. 20.

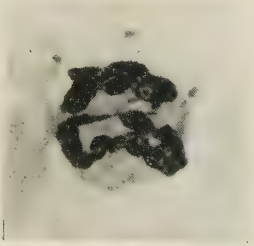


Fig. 21.

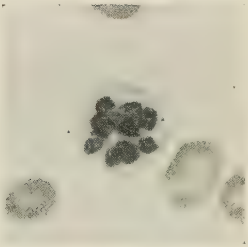


Fig. 22.

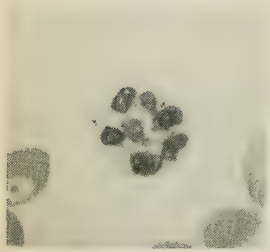


Fig. 23.

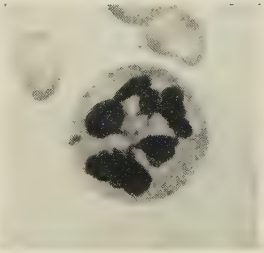


Fig. 24.

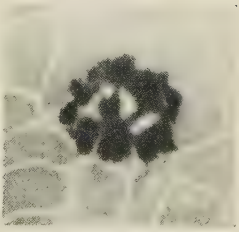


Fig. 25.

Fig. 17.—Macropolycytes (Type II), Megakaryocyte type. × 1000. Diameters, lower right, 20 μ; upper left, 17·5 μ.

Fig. 18.—Macropolycyte, Megakaryocyte type. × 1000. Diameter, 21 μ.

Fig. 19.—Macropolycytes, Megakaryocyte type. × 1000. Diameters, upper, 20·5 μ; lower, 15 μ.

Fig. 20.—Macropolycyte, Megakaryocyte type with basophil myelocyte. × 1000. Diameter, 20 μ.

Fig. 21.—Macropolycyte, Megakaryocyte type. × 1000. Diameter, 20 μ.

Figs. 22 and 23.—Polymorphs from a case of pernicious anæmia showing hypersegmentation of the nucleus. × 1000. Diameters, 12·5 μ.

Fig. 24.—Macropolycyte suggesting a mixed type of Type I and the Megakaryocyte type. × 1000. Diameter, 19 μ.

Fig. 25.—Macro-mast cell showing unusual type of nucleus, approximating to the Megakaryocyte. × 1000. Diameter, 18 μ.

Photomicrographs by C. F. Hill *and* W. E. Cooke.

whose duty is the destruction and elimination of old leucocytes.

This hypothesis is possibly true up to a point, and would account for the presence in pernicious anæmia of the hypersegmented nucleus in the polymorph of normal size as figs. 22 and 23.

The macropolycyte, however, is morphologically so distinctive, and occurs under such definite known conditions, that it is inconceivable that mere ageing could account for its presence. The conditions under which it appears are accompanied by the presence of myelocytes in the blood-stream, and from consideration of experimental work in other conditions we incline to the view that the dysleucopoiesis is due to local or general biochemical abnormalities acting upon the hæmocytoblast itself.

In order that the argument may be quite clear, it is necessary to summarise briefly the normal potentialities of the hæmocytoblast, the cell from which the true polymorph is derived.

The true neutrophil polymorph is quite a recent cell in the vertebrate scale. It is not seen in amphibia, fishes, reptiles, nor in any cold-blooded vertebrate. Only when we come to the birds is the homologue of the human neutrophil polymorph found.

The embryology is briefly as follows:

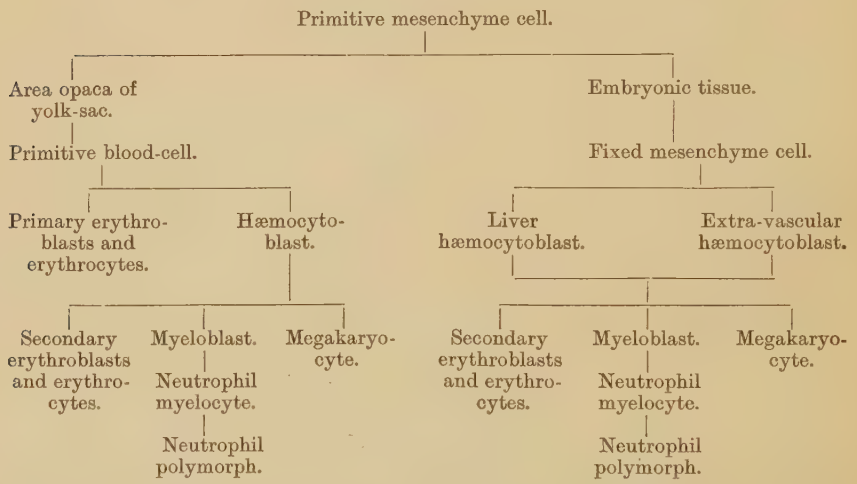
The primitive mesenchyme cells in the area opaca of the yolk-sac multiply, the peripheral cells become differentiated to form the endothelium of the vessels and the plasma, and the central cells form the primitive blood-cells. These blood-islands are, of course, extra-embryonic.

At the same time the embryonic mesenchyme is being differentiated into endothelium and plasma, but the blood-vessels and heart contain very few, if any, primitive blood-cells.

By the third week communication has been estab-

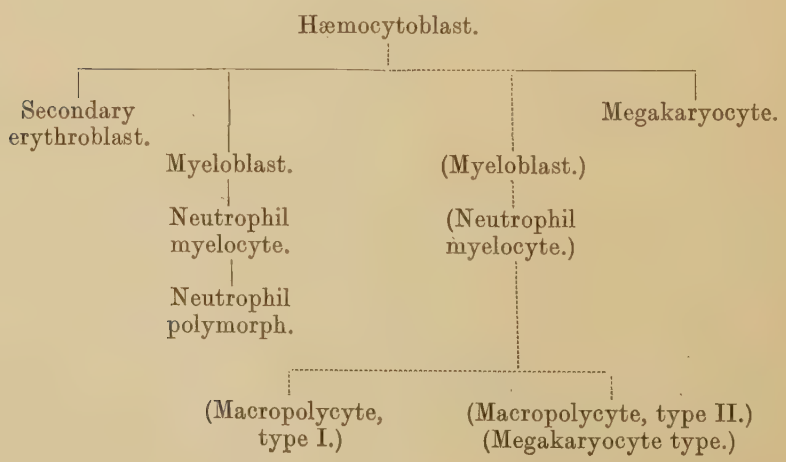
lished between the extra-embryonic vessels and the vessels of the embryo, and the first blood-cells of the embryo arise from invasion by the cells of the blood-islands of the yolk-sac. Diagrammatically, the fœtal origin of the polymorph may be represented as follows:

DIAGRAM I.



The post-natal origin of the neutrophil polymorph may be represented as follows:

DIAGRAM II.



The reason for mentioning this short scheme is that it is necessary at the outset to remember that the potentiality of the daughter cells of the hæmocytoblast under ordinary conditions is definite, and when differentiation has reached the myeloblast stage it is irreversible, development going on to the normal granulocyte of the blood-stream. The dotted lines may be left out of consideration for the time being.

Danchakoff concludes from her work on the development of the splenic anlage and other researches, that the factors which govern the potentialities of the hæmocytoblast lie in the conditions of environment, and alteration of that environment leads to alteration in the line of differentiation. This opinion is held by most other workers.

The results of tissue cultures *in vitro* are suggestive.

Lewis (M. R.) found that giant cells are formed from monocytes in hanging drop preparations of frog's blood, and Barta (E.) found that giant-cell formation took place in cultures of lymph nodes, at some distance from the surface. He ascribed this to oxygen deficiency, and found the addition of embryonic extracts diminished the effects of oxygen want, when the cells in the deeper layers behaved like those on the surface.

There are many factors in experimental conditions and disease in addition to simple oxygen want. Alteration in pH, gaseous diffusion, surface tension, saline ratios, N.P.N. retention, and lipoid changes must take place, and also influence cell metabolism, and possibly alter potentiality.

When we look for a specific disease in which there is evidence of dysfunction of the hæmocytoblast, we find such a case in pernicious anæmia. On the red-cell side we have the production of megalocytes, poikilocytes, and microcytes, and evidence of reversion in the presence

of the ichthyoid megaloblast. In the granulocyte series the evidence of dysfunction is also marked, and here the polymorph behaves as in no other disease. In pernicious anæmia there are frequently large numbers of cells of Classes IV. and V., as comparison between the normal polynuclear count and the count in that disease shows, and the nuclei are, in comparison with the normal nucleus, amblychromatic. In Class V. are placed cells with five and more nuclear segments. Polymorphs with 6, 7, 8, and even 10 segments, are not infrequent.

Figs. 22 and 23 are typical polymorphs showing the hypersegmented nucleus in normal-sized cells in pernicious anæmia. The following are polynuclear counts in cases of pernicious anæmia as soon as they came under observation :—

Normal.

I.	II.	III.	IV.	V.
10	25	47	16	2

Pernicious Anæmia.

I.	II.	III.	IV.	V.
8	20	39	22	11
6	27	40	22	5
7	20	44	24	5
7	16	29	39	9
20	31	17	16	16
16	22	32	16	14
10	15	38	24	13
12	34	26	20	8
6	20	42	20	12

The polynuclear counts are right-handed, a very marked contrast to the counts obtained in infective states, as the figures on p. 33 show.

The reaction of the marrow to infection is also

abnormal. Some cases of Addison's anæmia react in apparently normal manner to massive infections such as lobar pneumonia, as in the case quoted on p. 34, which we briefly repeat here.

F., Male, aged 36 years.

18/3/1926.

Hæmoglobin	27 per cent.
Erythrocytes	1,300,000 per c.mm.
Leucocytes	3000 ,,
Colour index	1·0.

Differential Count—

Polymorphs	48 per cent.
Lymphocytes	34 ,,
Monocytes	10 ,,
Myelocytes	8 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
10	15	38	24	13

On 5/4/1926 the patient developed acute lobar pneumonia, and on 8/4/1926 his leucocyte count was

Total leucocytes	28,600 per c.mm.

Differential Count—

Polymorphs	87 per cent.
Lymphocytes	12 ,,
Monocytes	0·5 ,,
Myelocytes	0·5 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
36	38	21	5	0

The patient died on 11/4/1926.

This was apparently an ordinary reaction to a massive pneumococcal infection, but there are two points in which it differs from the normal. The first is, that the

polynuclear count is not dislocated to the left as greatly as would be the case in a normal individual. Secondly, the polymorph nuclei, instead of being densely staining, showed marked deficiency in chromatin.

The next case had all the clinical signs of a massive infection, rigors, temperature 102° to 104° F., pus and *B. coli* in the urine, but there was no evidence of any marrow reaction.

Hæmoglobin	20 per cent.
Erythrocytes	800,000 per c.mm.
Leucocytes	4600 ,,
Colour index	1·2.

Differential Count—

Polymorphs	54 per cent.
Lymphocytes	38 ,,
Monocytes	2 ,,
Myelocytes	6 ,,

Polynuclear Count—

I.	II.	III.	IV.	V.
20	31	17	16	16

The patient died on 9/11/1926.

The one common feature in the two cases was the deficiency of chromatin in the nucleus of the polymorph.

In pernicious anæmia the cytoplasm of the polymorph sometimes shows basophil granules in addition to the usual oxyphil granules. The megakaryocyte type of macropolycyte has oxyphil and azurophil granules. The other cells of the granulocyte series show pathological changes. Dwarf forms are frequently present—micropolycytes, micro-eosinophils, and micro-basophils.

Added to the foregoing facts, we have evidence of reversion to the fœtal state in the production in the liver of megaloblasts and cells resembling megakaryocytes.

We can at least suggest that the surprisingly good

results obtained by Walterhöfer and Schramm by irrigation and scraping of the marrow cavities of the long bones in pernicious anæmia are due to the production of an altered local chemical state which improves for the time being the environment of the hæmocytoblast.

To complete the argument in favour of altered potentiality of some hæmocytoblast in the production of macropolycytes, reference to the other granulocytes of the blood-stream must be made.

In pernicious anæmia, and in infections accompanied by an eosinophilia, abnormal eosinophils are common. The micro-eosinophil as small as 5 microns in diameter occurs, and larger cells showing much more segmentation in their nuclei than normal are very frequent. The large eosinophils have as many as six or seven nuclear segments, and the cell-body may measure up to 16 microns in diameter.

Lastly, the mast cell is sometimes found behaving as if its parentage was in doubt.

Normally, this cell is found in 60 per cent. of people to the extent of about 0·3 per cent. of white cells. It measures 8 to 10 microns in diameter, and has a rounded or clover-leaf nucleus. Occasionally, in cases of marrow stress, one sees a giant, with a polymorphous nucleus.

Fig. 25 is a macro-mast cell found in the case of broncho-pneumonia, having the following white-cell count:—

Total leucocytes . .	33,600 per c.mm.
Polymorphs . . .	86 per cent.
Mast cells . . .	0·1 ,,

Occasional myelocytes were present in the blood-films.

Polynuclear Count—

I.	II.	III.	IV.	V.
42	37	17	4	0

All these facts point to dysfunction of the parent cell.

Wright has shown the precursor of the megakaryocyte of the marrow to be, in the embryo guinea-pig, a circulatory cell, and he looks upon the megakaryocyte as representing a blood-stream cell in the ancestry of mammals. A glance at Diagram I. shows, too, that the megakaryocyte or its precursor must be originally a circulatory cell.

Looking at Diagram II. again, and assuming the dotted lines represent potentiality in addition to morphological characters, we have what we think is the correct explanation of the genesis of the macropolycyte.

The hæmocytoblast under altered conditions of environment, in the diseases in which macropolycytes occur, has, to a lesser or greater degree, an altered potentiality, and gives to some myeloblasts a duplex biophore, mostly leucocytic, but partly also megakaryocytic, which shows itself either in the first type of macropolycyte if the bias is leucocytic, or with megakaryocytic bias in the megakaryocyte type of macropolycyte.

Prognostic Significance.—The first type of macropolycyte, that found in pyogenic infections, pernicious anæmia, etc., in association with myelocytes in the blood-stream, is, we think, of no prognostic value, except as a sign of extreme marrow stress—an index of altered biochemical constitution of the environment of the hæmocytoblast.

Type II., the megakaryocyte type, is of grave prognostic import, and in our experience no patient has lived beyond a few days from its appearance in the peripheral blood.

APPENDIX I.

STAINING METHODS.

Although of all methods Heidenhain's iron alum method is the most generally applicable, certain other stains may be used with advantage under special circumstances.

1. **Carbol-pyronin green.**—This stain is made up as follows :

Methyl green . . .	0·15	gm.
Pyronin	0·5	,,
Alcohol, 95 per cent. . .	15·0	c.c.
Carbolic water, 3 per cent. to .	100	,,

The films are dried in air, and the stain applied for ten or fifteen minutes. After washing and drying, they are examined, mounted or unmounted. No preliminary fixation is required. The stain imparts a deep blue colour to the polymorph nuclei and a pink to the cytoplasm ; the red cells stain feebly pink. Bacilli or cocci stain red ; the tubercle bacillus stains an intense red.

This is the stain *par excellence* for experimental work in which the phagocytic activity of the various classes of polymorph is to be observed, for it stains even tubercle bacilli without injuring the cytoplasm of the cells. It is also very useful for general work, and may be applied to the cells of any of the commonly used animals, with the exception of the dog and the cow, with excellent results. Further, it has the advantage that the time required for staining is short ; changes in the Arneth count may therefore be followed from hour to hour on films stained with carbol-pyronin green where they cannot on films stained with iron hæmatoxylin.

In certain abnormal conditions, such as in hæmorrhagic anæmia in the rabbit, the nucleus of the polymorph becomes altered in its staining properties, so that differentiation in iron alum is not easy to carry out. In such cases carbol-pyronin green will always prove satisfactory, although it is as well to increase the period of staining up to thirty minutes.

2. **Eosin-methylene Blue.**—This is an excellent stain for the polymorphs of most experimental animals, and also for the cells of man. It is unfortunately difficult in use. The film should be fixed in saturated corrosive sublimate for about fifteen minutes, washed, and then dipped into a 75 per cent. alcohol. It is next placed in a saturated solution of eosin in 75 per cent. alcohol for one minute, washed in water, and then immersed in a 1 per cent. methylene blue in water. In this it is stained for fifteen minutes or longer. A final washing completes the process.

Toluidine blue may be substituted for methylene blue with advantage. The method is very satisfactory in expert hands, and may be applied to films on coverslips as well as on slides.

Properly stained films show a deeply stained nucleus and finely stained eosinophil granules in the polymorph cytoplasm. The incomplete eosin staining is a necessity, for in many animals (especially the rabbit) the cytoplasmic granules are large and liable to obscure the nuclear convolutions if deeply stained. The method is ideal for the study of the polymorphs in the bone marrow; it is unsatisfactory for the polymorphs of the dog and the cow.

3. **Crystal Violet.**—The stain is made up as follows:—

Aniline water	50 c.c.
Sat. sol. crystal violet in 75 per cent. alcohol .	25 „
1 per cent. HCl in 75 per cent. alcohol . .	25 „

The film should be first fixed in corrosive sublimate for about fifteen minutes, and then dried. The stain is applied to the slide, and the slide warmed until fumes begin to rise; this takes from thirty seconds to one minute. A final washing completes the process. The customary differentiation with 10 per cent. acetic acid (as in staining for mitochondria) is better omitted, since it is desirable to have the nuclei of the polymorphs intensely stained.

With this method there is no difficulty in determining the nuclear configuration. It has, however, no special advantages except its simplicity in use and the short time required for its performance.

4. **Pappenheim's Panoptic Stain.**—This is to be recommended for the polymorphs of the dog and cow, in which the nuclear configuration is exceedingly complex.

The film is first fixed in corrosive; on this step the satisfactory results obtained from the method depend. For exactly three

minutes the film is stained with Jenner's stain, ten drops being placed on the slide. The same number of drops of distilled water is added, and the whole of the fluid drained off the film immediately; without washing, the film is then covered with a freshly prepared Giemsa, diluted 1 in 10 with distilled water. The staining is allowed to go on for ten minutes. After thorough washing the film is dried at room temperature.

This method proves satisfactory for the study of the nuclear configuration of the polymorphs in the bone marrow.

APPENDIX II.

THE NORMAL POLYNUCLEAR COUNT.

To determine the normal polynuclear count in man, and the extent to which it varies in health, we have taken the counts for ninety normal individuals, each of whom was carefully examined to exclude the presence of septic foci, etc.

The figures for each of these ninety counts, together with the values for the weighted means, are given in tabular form below.

	I.	II.	III.	IV.	V.	Mean.		I.	II.	III.	IV.	V.	Mean.
1	10	17	56	15	2	2·82	21	17	26	44	12	1	2·54
2	13	27	53	7	0	2·54	22	7	29	55	6	3	2·69
3	16	16	54	10	4	2·70	23	14	24	54	8	0	2·56
4	9	21	53	16	1	2·79	24	8	33	49	7	3	2·64
5	12	25	52	9	2	2·64	25	14	18	55	12	1	2·68
6	7	29	47	16	1	2·75	26	7	29	47	14	3	2·77
7	14	24	45	16	1	2·66	27	13	27	48	12	0	2·59
8	8	20	51	18	3	2·88	28	17	21	48	10	4	2·63
9	9	30	53	8	0	2·60	29	5	18	49	25	3	3·03
10	7	27	57	9	0	2·68	30	15	23	43	16	3	2·69
11	18	23	48	10	1	2·53	31	14	22	40	21	3	2·77
12	6	27	36	27	4	2·96	32	14	33	44	9	0	2·48
13	18	22	44	14	2	2·60	33	10	18	55	15	2	2·81
14	15	23	51	11	0	2·58	34	10	21	48	17	4	2·84
15	4	22	47	23	4	3·01	35	7	20	47	22	4	2·96
16	9	23	51	14	3	2·79	36	7	20	47	22	4	2·96
17	16	22	55	7	0	2·53	37	10	19	46	23	2	2·88
18	15	34	40	11	0	2·47	38	3	31	46	18	2	3·05
19	19	24	46	10	1	2·50	39	13	24	42	18	3	2·74
20	16	20	45	18	1	2·68	40	12	28	44	13	3	2·67

	I.	II.	III.	IV.	V.	Mean.		I.	II.	III.	IV.	V.	Mean.
41	10	27	48	13	2	2·70	66	7	31	40	20	2	2·79
42	7	28	45	14	6	2·84	67	12	30	46	12	0	2·58
43	6	18	45	27	4	3·05	68	9	24	47	17	3	3·11
44	4	23	48	22	3	2·97	69	11	29	43	16	1	2·67
45	7	20	42	26	5	3·02	70	12	28	47	11	2	2·63
46	8	26	47	18	1	2·78	71	9	18	55	16	2	2·84
47	6	21	46	23	4	2·98	72	11	28	45	15	1	2·67
48	9	30	38	21	2	2·77	73	7	24	43	21	5	2·93
49	11	27	48	14	0	2·65	74	8	19	51	19	2	2·85
50	11	25	47	16	1	2·71	75	7	29	50	14	0	2·71
51	6	28	47	17	2	2·81	76	9	34	44	12	1	2·62
52	17	20	46	14	3	2·66	77	9	27	49	13	2	2·72
53	9	26	42	19	4	2·83	78	8	30	39	21	2	2·79
54	7	30	40	19	4	2·83	79	7	28	39	24	2	2·86
55	12	25	44	15	4	2·74	80	10	29	39	20	2	2·75
56	12	27	38	20	3	2·75	81	6	27	45	20	2	2·85
57	11	29	44	14	2	2·67	82	8	25	48	16	3	2·81
58	12	29	46	12	1	2·61	83	3	23	49	21	4	3·00
59	8	21	47	22	2	2·89	84	11	24	45	16	4	2·78
60	11	25	47	16	1	2·71	85	6	30	44	18	2	2·80
61	11	27	45	12	5	2·73	86	11	31	42	14	2	2·65
62	6	19	52	20	3	2·95	87	8	22	48	18	4	2·88
63	9	23	44	22	2	3·08	88	12	29	40	15	4	2·70
64	12	25	43	17	3	2·74	89	12	28	42	15	3	2·69
65	4	23	49	20	4	2·97	90	6	20	48	22	4	2·98

Taking this series as representative of the counts of normal persons, we can draw from it the following conclusions :

1. The average weighted mean in health is 2·74, this corresponding to a count of

I.	II.	III.	IV.	V.
12	25	44	15	4

It is interesting to observe that while this series of normals gives the average weighted mean as 2·74, the series published in the first edition of this book gives 2·70. These figures are virtually the same, and virtually the same as the figure calculated from the normal count as originally laid down by Arneth himself, which is 2·76.

2. The highest extreme among the weighted means is 3·11, corresponding to a count of

I.	II.	III.	IV.	V.
9	24	47	17	3

while the lowest extreme is 2·47, corresponding to

I.	II.	III.	IV.	V.
15	34	40	11	0

Between these extremes the distribution is represented in fig. 26 (*a*) ; it is roughly symmetrical, with a standard deviation of 0·18.

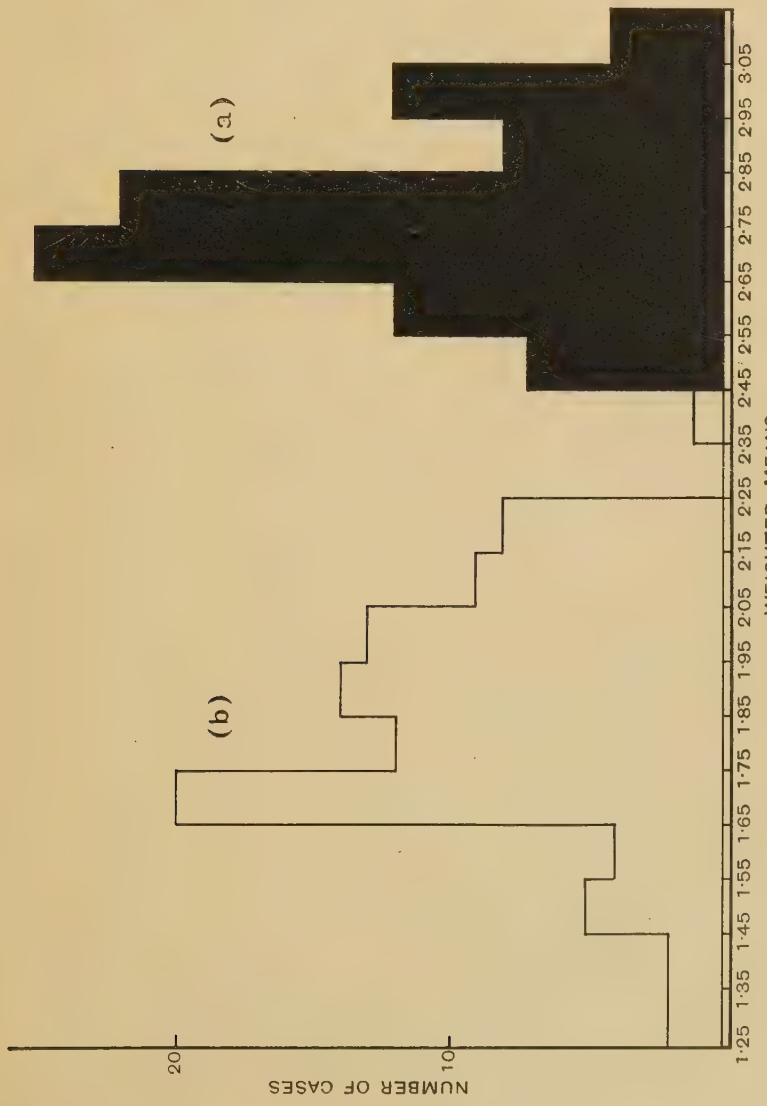


FIG. 26.

Frequency polygons for (*a*) 90 normal cases (dead black), and (*b*) 90 tubercular cases. Weighted means grouped in intervals of 0·1.

3. As a test for the normality of any particular count, we can accordingly lay down the rule that any showing a weighted mean of less than 2·45 or of more than 3·15 is certainly outside the range of normal variation. For most purposes we are satisfied that this rule is sufficient, and as an illustration of how well it holds, we show in fig. 26 (*b*) the distribution of the weighted means of the first ninety cases of tuberculosis given in the first edition of this book. The upper extreme of this distribution is 2·42, which is just outside the normal range ; the mean is 1·82, and the lower extreme actually as low as 1·31. Every one of these tubercular cases accordingly shows an abnormal count, as judged by the proposed rule. Further, by the same rule we could say that the count,

I.	II.	III.	IV.	V.
6	17	40	25	12

as obtained in a case of pernicious anæmia, is outside the normal limits, for it gives a weighted mean of 3·20.

While this rule is quite sufficient for all practical purposes, there may always arise a case like this. Suppose we find in a certain individual the count

I.	II.	III.	IV.	V.
15	34	40	11	0

with a mean of 2·47. Applying the rule, we should say that this count is a normal one, for its mean is above 2·45. It is, however, only just within the normal limits, and it is impossible to overlook the fact that, had we seen the individual in perfect health, his count might have been

I.	II.	III.	IV.	V.
9	23	44	22	2

with a mean of 3·08. Had this been the case, and we cannot say that it was not, the mean of 2·47 would represent a considerable deflection ; at the same time it is also possible that this mean of 2·47 is normal for the particular individual.

One guide in a case like this is to consider the probability of a normal mean of this value occurring, and so we tabulate below the probability of normal means falling between certain limits, on the assumption that the series of normal counts given above is perfectly representative.

Probability of a mean lying between	P.
2·45 and 2·55	0·077
2·55 „ 2·65	0·133
2·65 „ 2·75	0·277
2·75 „ 2·85	0·244
2·85 „ 2·95	0·088
2·95 „ 3·05	0·133
3·05 „ 3·15	0·044

In this particular case, accordingly, the probability that the mean 2·47 is a normal one is 0·077 (about a 1 in 13 chance), and the probability that it represents a left-handed deflection, small or great, is 0·922, or very nearly a certainty. If this deflection exists, the probability that it is greater than 0·1 (on the mean) is 0·79 approximately, and that it is greater than 0·2 is only about 0·5, or evens. There is a 4 to 1 chance that the deflection is not greater than 0·3, and so we might conclude that (*a*) the mean of 2·47 is not likely to be a normal mean, but that (*b*) the deflection which is present is unlikely to be greater than about 0·25.

If the count to be considered lies below the normal range, *e.g.*

I.	II.	III.	IV.	V.
33	35	28	4	0

with a mean of 2·03, the same sort of problem arises. This mean is admittedly abnormal, but is the normal mean 2·45, 2·74, or 3·15, all of which are possible ? If the normal mean is the first figure, the deflection is small (0·42) ; if the normal mean is 2·74, the deflection is much greater (0·71) ; and if 3·15 is the normal mean the deflection, which would then amount to 1·12, is very great indeed. Here again we can only say that the chances are even that the normal mean lies between 2·65 and 2·85, and that the chances are thus even that the deflection is about 0·7. There is about 4 to 1 against the deflection being either greater than 0·8 or less than 0·6, and more than 20 to 1 against it being greater than 1·0.

While these facts and this method of consideration should be borne in mind, the best practice is, if possible, to repeat the doubtful count at short intervals, so as to find out whether it is stationary or

moving to the left. A few weekly counts as a rule completely clear up any dubiety.

One further point remains to be mentioned. Inspection of the series of normal counts will show that not one contains a double maximum. Both experience and theoretical considerations go to show that counts with such double maxima as

I.	II.	III.	IV.	V.
33	25	35	6	1

in which the figures for Classes I. and III. exceed the intervening figure for Class II., are indicative of an unsteady state. In experimental work these double maxima almost invariably occur during the recovery from a left-handed deflection; in clinical practice they are most common in cases of improving infections, and especially in rapidly healing tuberculosis. Even so, they are found in only about 3 per cent. of cases; it would be going too far to say that they constitute a favourable sign, but they at least indicate a recovery of the count towards normal. Examples will be found in the first edition of this book, and in experimental papers.

Since the rabbit is the principal experimental animal for work on the polynuclear count, mention of the normal count in that animal will not be out of place. The average value for the weighted mean is, in our experience, 2·77, and much the same as in man; the variation, however, is considerably greater. Values for the mean are met with up to 3·40, and values as low as 2·30 are not exceptional; whether this is due to there being a greater intrinsic variation in the count, or whether it is due to the difficulty of being certain as to perfect health in an animal so liable to infection is difficult to say. We make a practice, however, of rejecting as abnormal animals with a weighted mean of less than 2·3. Double maxima occur in the rabbit, and are definitely a sign of instability in the count.

Kennedy has attempted to define the normal polynuclear count for the rat, but has found even more variation than in the rabbit. The average mean is about 2·4.

Satisfactory determinations of the normal count in other animals are wanting, and would constitute an excellent if somewhat tedious line of research. Normals certainly exist for all the animals which possess either polymorphs or their homologues, and even in the reptilia a polynuclear count can be carried out on the polymorpho-

nuclear eosinophiles. We have ourselves investigated what corresponds to the polynuclear count in the frog, and have found a very constant normal state,

I.	II.	III.	IV.	V.
50	40	10	0	0

(mean 1·60), which, however, displays certain seasonal alterations.

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